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MODIFICATION OF THE FREQUENCY OF CHROMOSOMAL REARRANGEMENTS INDUCED BY X-RAYS IN DROSOPHILA. I. USE OF NEAR INFRARED RADIATION

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STUDENTS of radiation genetics have outlined in recent years the more general aspects of the phenomenon whereby the activating energy of ionizing radiations induces changes within the chromosome that result in its eventual breakage. From the physical standpoint, information is available concerning the sequence of phenomena that lead to the activation of groups of atoms that presumably effect such severance. Comparison of the effects of radiations producing vastly different densities of activations within small areas suggests that the process of chromosome breakage depends ordinarily on a series of "ionizations" (LEA and CATCHESIDE 1942). The amount of energy required to disrupt the chromosome may accordingly be calculated, but the sequence of molecular changes by which this end is attained remains unknown.

Analysis of suitable biological material has revealed various types of alterations resulting from the rearrangement of parts of broken chromosomes; in this procedure the positions of the induced breaks have been plotted. Observations made on the salivary-gland chromosomes of Drosophila indicate that the breaks occur between bands rather than within the bands. The position of the break is revealed because the broken ends have recombined with other similar ends. Thus, the process of disruption by X-rays involves a temporary separation of those bonds that normally serve to maintain the linear continuity of the chromosome. The experimentally induced recombination types show such patterns of realignment and duplication of parts of chromosomes as presumably have occurred in phylogeny, but the methods used in their production have not provided a clear understanding of the sequences by which these changes have occurred in nature. In short, they have given clues, but not an answer to the problem of spontaneous chromosomal alteration.

Certain aspects of the cycle of chromosome breakage and recombination have been elucidated by altering the conditions, such as temperature and rate of treatment, under which the ionizing radiation was delivered. Resulting differences in the proportions of chromosomal derangements have been attributed to differential effects on the processes of chromosome restitution and recombination (summaries of earlier studies, chiefly on plant materials, presented by SAX 1941; LEA and CATCHESIDE 1942; DARLINGTON and LA COUR 1945). On theoretical grounds it appears possible, therefore, to modify the capacity for

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reattachment of the bonds uncoupled by ionizing radiation if supplementary treatment is given prior to the time that new combinations are established. In order that breakage and reattachment may be separated in time, the biological assay should be made on organisms in which no restitution or recombination occurs during irradiation. Chromosomes that are capable of shifting their positions within the nucleus, as those of actively dividing cells, are presumably able to establish new contacts, even during the period of irradiation, but the chromosomes of Drosophila are evidently immobile within the spermatozoon, available evidence indicating that they do not combine to form new rearrangements until after the irradiated sperm has penetrated the egg in the process of fertilization (MULLER 1940; KAUFMANN 1940, 1941). Since irradiated males may be kept for several days before copulation, a considerable period of time is available between irradiation and fertilization for efforts to alter experimentally the capacity of the regions of breakage to recombine or to undergo restitution.

Any supplementary treatment that is to be effectively measured must supply sufficient energy to promote chemical activation within those regions sensitized by the ionizing radiations, without in itself being capable of inducing chromosomal rearrangements. There is some background of experience for anticipating effective interaction of two activating sources of energy. Beginning with BOVIE's study (1913) of the effect of ultraviolet radiation and heat on egg albumin, several investigators have shown that an increased sensitivity to heat follows ultraviolet treatment of living cells (BOVIE and DALAND 1913 on Paramecium; CURRAN and EVANS 1938, on bacteria; ANDERSON and DUGGAR 1941, on yeast). Various other studies of combination treatment have been reviewed by PRAT (1933) and BĚLEHRÁDEK (1935). In a series of studies, the first of which is here reported, an attempt was made to measure the effect on chromosome breakage and recombination of superimposing near infrared and ultraviolet irradiation on X-ray treatment.

Various types of evidence suggest that in Drosophila the direct effect of the ionizing radiation is the production of potential rather than actual breaks in the chromosomes. There is also considerable evidence that regions of potential breakage that do not participate with other similar regions to form new combinations may have the original sequence of parts restored. The effect of supplementary treatment may be detected, therefore, if it alters the capacity of the regions of potential breakage for restitution or recombination. Any factor promoting restitution prior to the time of recombination will decrease the number of potential breaks, and increase thereby the frequency of sperms transmitting in fertilization an unaltered complement of chromosomes. On the other hand, any supplementary treatment effective in disrupting the chromosome and "sealing" the broken ends, so that they are incapable of subsequent union, will result in centric chromosomes lacking terminal sections necessary for normal development of the embryo. Such inviable combinations would be eliminated, so that the proportion of detectable alterations would be reduced. In either case, supplementary treatment that is effective at the molecular level

should be reflected by a reduction in the number of altered sperms and in the complexity of chromosomal rearrangements as compared with the controls treated only with the ionizing radiation.

Experiments in which ultraviolet radiation of wave length 2537 Å was used after X-rays (KAUFMANN and HOLLAENDER 1942, 1946) indicate that the supplementary treatment is effective in eliminating some of the regions of potential breakage. Another set of experiments, herein reported, was designed to measure the effects of near infrared irradiation when superimposed on the X-ray treatment. In the course of the work, clues were obtained which suggested that the treatment with near infrared might be capable of sensitizing the chromosomes to subsequent breakage by X-rays. The results of experiments along these lines also form a part of the present report.

EXPERIMENTAL METHODS

The X-ray treatment was given by using a Universal type Coolidge tube, with a tungsten target, operating at about 85 kilovolts and 5 milliamperes. The dosage was measured by a Fricke-Glasser dosimeter, manufactured by the Victoreen Instrument Company.

The method of exposure to the near infrared radiation was conditioned by the requirements of the living material; the limits of tolerance of the flies to high temperatures had to be considered, and a type of apparatus designed that would operate within these limits. A commercial infrared drying lamp (250 watts) was used in conjunction with a pair of plano-convex condensing lenses and two filters. A saturated solution of iodine in carbon tetrachloride in a layer about 3 cm deep, in a glass battery jar 4×15×30 cm served as one of the filters. A glass cooling coil with circulating faucet water was placed in the battery jar so that the center part of the jar was unobstructed; this prevented the filter from becoming a secondary radiator. The other filter, of Corning glass 1½ mm thick (#242, new 2404), was used to absorb the residual longer infrared rays; it was attached to the battery jar in order to utilize the cooling properties of the enclosed coil. Additional details will be presented in another publication. The spectral energy distribution (incident and transmitted) is given in figure 1.

The method of biological assay involved cytological analysis of the salivary-gland chromosomes of the F_1 larval progeny of the irradiated fathers. Following treatment, the Oregon-R males used in these experiments were mated with virgin females of the same stock. Salivary glands secured from third-instar larvae were prepared for study by fixing and staining in acetic orcein.

Much valuable assistance was provided in the course of these experiments by JEAN LANE HEROLD and MAYDELLE BISHOP STEWART; their contributions are acknowledged with thanks. We are also indebted to DR. U. FANO for his counsel, particularly with regard to statistical methods.

Some of the results have been presented in fragmentary form in preliminary statements (KAUFMANN and HOLLAENDER 1945; and in Year Books Nos. 41 and 42 of the CARNEGIE INSTITUTION of WASHINGTON for 1942 and 1943).

EFFECT OF POSTTREATMENT WITH NEAR INFRARED RADIATION

In the first experiments, 4000 roentgens of X-rays were given in two equal fractions at 16-day intervals (at Cold Spring Harbor), with exposure of the flies in the intervening period to near infrared radiation for either 72, 144, or 216 hours (at Bethesda). Males remained with the females for the following three days, and were then discarded. Thus a maximum of 19 days elapsed be-

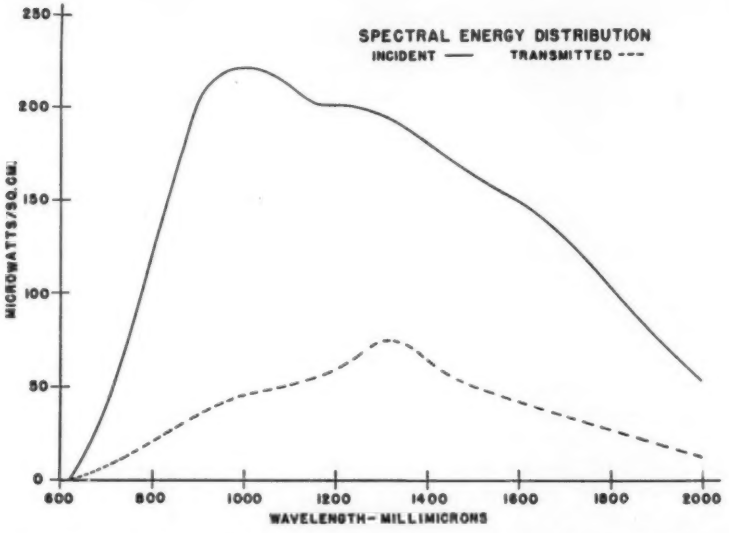

FIGURE 1.—Spectral energy distribution of radiation from a 250 watt infrared lamp after it has passed an iodine-saturated carbon tetrachloride solution and a Corning glass filter #242. Solid line = incident radiation; broken line = radiation transmitted by the epidermal cuticula. All measurements were made with the Beckman spectrophotometer especially adapted for these tests.

tween the first X-ray treatment and the last transfer of the treated sperm by the males. The percentages of altered sperms and of breaks, detected by salivary-gland-chromosome analysis, are indicated in table 1, together with data for two groups of controls, one of which received only near infrared treatment, the other only 4000 roentgens of X-rays. (The X-rayed controls included some given 4000-r single treatment as well as some treated with two fractions of 2000 r each, since, as was reported by KAUFMANN (1941), the frequencies and types of detectable rearrangements do not differ significantly if the X-ray dosage is administered continuously or in a series of fractions.)

The near infrared radiation when given alone induced no chromosomal aberrations—thereby meeting the primary qualification indicated on page 350 and was effective, when intercalated between the two fractions of X-rays, in reducing the frequency of detectable chromosomal aberrations in proportion to

the time of treatment. With respect to the frequency of altered sperms, the groups differ significantly from each other and from the 4000-r control, except for the 72-hour sample when compared with the 144-hour sample and the controls, and the 216-hour sample when compared with the 144-hour group. Following the 216-hour exposure, the values obtained are only slightly higher

TABLE 1

Frequency of detectable chromosomal rearrangements obtained following 4000 r of X-rays plus near infrared treatment.

TREATMENT: X-RAY IN ROENTGENS; INFRARED IN HOURS	TOTAL SPERMS TESTED	REARRANGEMENTS IN SPERMS TESTED		% SPERMS SHOWING REARRANGEMENTS	NO. OF BREAKS DETECTED	BREAKS PER 100 SPERMS	MEAN BREAK NUMBER
		ABSENT	PRESENT				
2000 r+72 hrs.+2000 r	149	109	40	26.85±3.63	99	66.44	2.48
2000 r+144 hrs.+2000 r	134	106	28	20.90±3.51	79	58.96	2.82
2000 r+216 hrs.+2000 r	78	67	11	14.10±3.94	25	32.05	2.27
4000 r alone*	203	140	63	31.03±3.25	178	87.68	2.83
170 hrs. alone	100	—	—		—		
4000 r controls cf. with 2000 r+216 hrs.+2000 r		χ^2=8.326		N=1	P=ca. .0035		

* Includes 2000+2000 as well as 4000-r single treatment. KAUFMANN (1941) has shown that comparable frequencies of rearrangements are obtained whether X-ray dosage is given continuously or in two or more fractions (cf. also MULLER 1940).

The frequencies for the X-ray "controls" are quite similar to those reported by BAUER, DEMEREC, and KAUFMANN (1938), who found 64/215 or 29.7±4.04 altered sperms, and 108/215 or 84.4 breaks per 100 sperms, with a mean break number of 2.88.

than the nine percent of altered sperms and the 24 percent of breaks to be expected (on the basis of previous studies) from treatment with the final fraction of 2000 r of X-rays. Are such results to be attributed to a direct effect of the energy supplied by the near infrared on the potential breaks induced by the first fraction of the X-ray treatment?

Before appraising the data that provide the answer to this question, consideration should be directed to those conditions that influence the frequencies of detectable chromosomal derangements following X-ray treatment of Drosophila sperm. The salivary glands selected for study represent a random sample of the individuals that survive to late larval stages. These samples provide altered and unaltered types in proportions that are duplicable, within the limits of the experimental error, in parallel experiments involving individuals of the same stock (compare, for example, the similar values obtained following 4000 r in these and other experiments; KAUFMANN 1941). It is important that care be taken to select only virgin females for mating with the treated males, and to use only larvae developing from eggs fertilized by spermatozoa that were mature at the time of treatment; otherwise the proportion of

unaltered glands may be increased greatly. To insure the use of sperm that was mature at the time of treatment, males were discarded within the period of time that past experience had indicated was necessary for the immature sperm to become functional. For actively copulating males of *D. melanogaster* this is not less than 12 to 15 days, as various workers beginning with HARRIS (1929) have shown; for males that had not previously mated, two or more copulations on the nineteenth day after treatment did not exhaust the old sperm (DEMEREC and KAUFMANN 1941).

The fact that treated spermatozoa may be retained for many days by unmated males was the basis for selecting the 16-day interval between the X-ray fractions represented in table 1. A previous study of the effects of fractionation of the total X-ray dose had shown that, despite the 16-day interval between two fractions of 2000 r, there was no reduction in the frequency of chromosomal aberrations as compared with the single-dose, 4000-r control (KAUFMANN 1941). In this newer work we were adding another variable—namely, several hours of exposure to near infrared rays during the 16-day period. Might higher temperatures induced within the body of the fly while exposed to the near infrared rays accelerate those processes that make available in copulation spermatozoa that were not mature at the time the first fraction of X-rays was administered?

A decisive answer could be obtained if inseminated females exposed to the near infrared beam would retain the sperm until after completion of the treatment. Accordingly, females inseminated by males which had received 4000 roentgens of X-rays were placed in the near infrared beam for periods of 72, 144, or 216 hours, in a series of tests (all completed at Cold Spring Harbor to avoid delay). Despite efforts to provide a medium that would be adequate to maintain the cultures but which would not be conducive to oviposition, all the females deposited eggs while exposed to the near infrared radiation, and in this process practically exhausted their store of spermatozoa. The limited number of pairs of glands obtained from larvae developing from eggs laid after the females were removed from the near infrared radiation was inadequate to provide an answer to the problem concerning the mode of action of these rays.

In the light of this experience it seemed advisable to extend the experiments in which males were treated, reducing the interval between X-raying and mating. Males were given 4000 r of X-rays, exposed thereafter for either 72, 144, or 216 hours to the beam of near infrared radiation, and then mated. Three days after mating the flies were transferred to new bottles; three days later the males were discarded and the females transferred to a third set of bottles. Thus the maximum period of time elapsing between the X-ray treatment and the last copulation was nine days for the first group, 12 days for the second, and 15 days for the third. Results of the cytological analysis of the salivary-gland chromosomes of the F_1 larvae are presented in table 2.

This analysis yielded percentages of altered sperms which do not differ significantly among the three groups nor from values obtained previously using 4000 roentgens of X-rays without supplementary treatment. It should be

pointed out that the 144-hour material includes a 34-break rearrangement (KAUFMANN 1943); if this unique case were eliminated (and no other rearrangement with half so many breaks has been found in this laboratory), the 144-hour material would occupy a position intermediate between the 72- and 216-hour groups with respect to frequency of breaks as well as of altered sperms.

Posttreatment with near infrared radiation does not seem to be effective, therefore, in eliminating the potential breaks induced by X-rays. Any effective action of near infrared rays in reducing the frequency of detectable chromosomal rearrangements in Drosophila (as shown in table 1) must accordingly be attributed to an accelerating effect on those processes that make available

TABLE 2

Frequency of chromosomal rearrangements induced by 4000 r of X-rays followed by exposure to near infrared radiation for 3, 6, or 9 days.

TREATMENT: X-RAY IN ROENTGENS; INFRARED IN HOURS	TOTAL SPERMS TESTED	REARRANGEMENTS IN SPERMS TESTED		% SPERMS SHOWING REARRANGEMENTS	NO. OF BREAKS DETECTED	BREAKS PER 100 SPERMS	MEAN BREAK NUMBER
		ABSENT	PRESENT				
4000 r+ 72 hrs.	182	130	52	28.57±3.35	139	76.37	2.67
4000 r+144 hrs.*	301	220	81	26.91±2.56	247	82.06	3.05
4000 r+216 hours	209	155	54	25.84±3.03	138	66.03	2.56
4000 r (from table 1)	203	140	63	31.03±3.25	178	87.68	2.83
4000 r and 4000 r+216 hrs.		χ^2=1.366		N=1	P=ca. .22		

* The 144-hour material includes a 34-break rearrangement (KAUFMANN 1943) which increases materially the percentage of breaks per total sperm. If this case were not included the percentage of altered sperms would be 26.67, the breaks per 100 sperms only 71.00, the mean break number 2.66.

for copulation sperm that was not mature at the time of the original X-ray treatment. Probably the mixture of the new sperm with the old begins between the ninth and the twelfth days in actively copulating males. This suggestion is derived from a consideration of frequencies obtained from larvae taken from the different culture bottles in which the males and females were kept for three days. For batches of 72-hour material tested in this way, the first and second transfers (three to six, and six to nine days after irradiation) were quite similar, furnishing respectively 27.0 and 27.7 percent of altered sperms; the 144-hour material gave 36.4 percent for the six-to-nine-day transfer, and 25.0 percent for the nine-to-12-day transfer; the 216-hour group, in which the males first mated on the ninth day, provided 30.3 percent for the nine-to-12-day interval, and 23.8 percent for the 12-to-15-day group. In all these groups the numbers were small and the errors correspondingly large, but the general trend confirms the interpretation that exposure to near infrared accelerates the changes by which immature sperm becomes mature.

EFFECT OF PRETREATMENT WITH NEAR INFRARED RADIATION

Although the near infrared radiation did not eliminate or modify the capacity for reattachment of the X-ray-induced potential breaks, there remains the question of its effect on chromosomes when used prior to X-rays. To avoid the complicating factors of a fractionated dose, the males were given a single treatment of 4000 r immediately following exposure to the near infrared. The controls, which had been stored at 18 degrees C while their brothers were in the infrared beam, were treated simultaneously with the X-rays. The males, and the females with which they had been mated, were moved at three-day intervals to new culture bottles until the males were discarded seven to nine

TABLE 3

Effects of treatment with near infrared radiation prior to X-raying sperm of Drosophila.

TREATMENTS: X-RAY IN ROENTGENS; INFRARED IN HOURS	FREQUENCY OF CHROMOSOMAL BREAKS						
	TOTAL SPERMS TESTED	REARRANGEMENTS IN SPERMS TESTED		% SPERMS SHOWING REARRANGEMENTS	NO. OF BREAKS DETECTED	BREAKS PER 100 SPERMS	MEAN BREAK NUMBER
		ABSENT	PRESENT				
72 hrs.+4000 r	105	59	46	43.81±4.84	116	110.48	2.52
4000 r alone	110	77	33	30.00±4.37	81	73.64	2.46
		χ^2= 4.409		N=1	P=ca. .034		
72 hrs.+4000 r	198	99	99	55.00±3.55	282	142.42	2.85
4000 r alone	102	71	31	30.39±4.55	80	78.43	2.85
		χ^2=10.341		N=1	P=ca. .0012		
24 hrs.+4000 r	136	72	64	47.06±4.28	186	136.76	2.91
4000 r alone	134	92	42	31.34±4.01	96	71.64	2.29
		χ^2= 6.994		N=1	P=ca. .0085		
Total combined treatment	439	230	209	47.61±2.38	584	133.03	2.79
Total 4000 r	346	240	106	30.64±2.48	257	74.28	2.43
		χ^2=23.195		N=1	P=<.0001		

days after irradiation. (This procedure parallels that used in previous studies to measure the effects of 4000 r on the chromosomes of spermatozoa.) The cultures were kept in the 18-degree coldroom for the entire period of embryonic and larval development.

Results of the cytological analysis in three separate experiments are presented in table 3. In two experiments the period of exposure to the near infrared radiation was 72 hours, and in the third only 24 hours. Data from the combination treatments and X-ray controls, which are paired in table 3, were derived from males that were exposed simultaneously to the X-rays in capsules lying side by side. It should be pointed out that the 72-hour treatment indicated in the first line of table 3 was administered with a different lamp and set of condensing lenses from that used for the other 72-hour, and for the

24-hour material. The frequencies of altered sperms obtained following the combination treatments are significantly higher than those of the controls in all three experiments, and the combined data show that the difference of the percentages is 4.9 times its standard error. (A test of independence by the χ^2 method also indicates that the probability is very low that the difference in frequency of rearrangement in the two samples is attributable to the sampling error.) These data suggest that pretreatment with near infrared radiation is

TABLE 4

Effect of pretreatment for one hour on X-ray-induced breakage of chromosomes of sperm of Drosophila.

TREATMENTS: X-RAY IN ROENTGENS; INFRARED IN HOURS	FREQUENCY OF CHROMOSOMAL BREAKS						
	TOTAL SPERMS TESTED	REARRANGEMENTS IN SPERMS TESTED		% SPERMS SHOWING REARRANGEMENTS	NO. OF BREAKS DETECTED	BREAKS PER 100 SPERMS	MEAN BREAK NUMBER
		ABSENT	PRESENT				
Near infrared (1 hr.)+4000 r	142	91	51	35.92±4.03	137	96.48	2.69
Infrared (unfiltered), temp. 39°C (1 hr.)+4000 r	140	88	52	37.14±4.08	129	92.14	2.48
4000 r (from table 3)	346	240	106	30.64±2.48	257	74.28	2.43
4000 r *cf.* with total 1 hr. treatment:		χ^2=2.427		N=1		P=ca. .12	

a method of increasing the yield of detectable chromosomal alterations in Drosophila.

The 72-hour and the 24-hour exposures were chosen arbitrarily. To determine whether a similar effect might be produced in a short time, males were exposed for one hour prior to a 4000-r X-ray treatment. (Inasmuch as the control values presented in table 3 were so similar to each other and to values obtained in previous work, no additional 4000-r controls were provided for this material.) The results are presented in table 4, together with a set of data obtained following exposure of males for one hour to the unfiltered radiation of the infrared lamp at a distance that provided a temperature of about 39 degrees C within the vials containing the flies. The frequencies of aberrations, which are intermediate between those induced by 4000 roentgens alone and those obtained in the experiments involving 24 hours and 72 hours of pretreatment, suggest that some effect of pretreatment may occur within a period of one hour. Data from these one-hour-pretreatment studies have been combined in table 5 with the 24-hour and 72-hour data for comparison with all of the 4000-r X-ray controls.

TYPES OF ALTERATIONS PRODUCED BY THE COMBINED TREATMENTS

Increased break frequency, as past studies have revealed, is a function of

increasing X-ray dosage. Values of the same order of magnitude as those produced by the near infrared pretreatment plus 4000 roentgens of X-rays had previously been induced by a dose of 5000 r. For example, BAUER, DEMEREC, and KAUFMANN (1938) analyzed a sample of 217 pairs of glands following a 5000-r treatment of sperm of *D. melanogaster*, and found 87 with rearrangements (40.09 ± 3.33 percent) involving 272 breaks (125.35 for each 100 sperms). In more extensive data secured for this dosage level, BAUER (1939) recorded 273 pairs of glands with aberrations out of a total of 608 examined (44.9 ± 2.02 percent), with 876 breaks (144.08 percent). An effort was made accordingly to

TABLE 5

Summarized data for pretreatment plus X-rays, and for X-rays alone.

TREATMENT: X-RAY IN ROENTGENS; INFRARED IN HOURS	FREQUENCY OF CHROMOSOMAL BREAKS						
	TOTAL SPERMS TESTED	REARRANGEMENTS IN SPERMS TESTED		% SPERMS SHOWING REARRANGEMENTS	NO. OF BREAKS DETECTED	BREAKS PER 100 SPERMS	MEAN BREAK NUMBER
		ABSENT	PRESENT				
Pretreatment+4000 r	721	409	312	43.27 ± 1.84	850	117.89	2.72
4000 r alone	549	380	169	30.78 ± 1.97	435	79.23	2.57
		$\chi^2 = 20.668$		N = 1	P = < .001		

determine whether pretreatment with near infrared radiation alters the proportions of simple and complex rearrangements induced by 4000 r so as to more nearly resemble those induced by 5000 r without pretreatment.

A comparison, using the χ^2 method, of the frequencies of two-break, three-break, and other multiple-break types, indicates that there are no significant differences between the 4000-r controls and the 4000-r pretreated material (table 6). A more rigid application of the test can be made by grouping the two-break, three-break, and four-break rearrangements on the one hand, and all the more complex rearrangements on the other. Following such grouping the test discloses an excess of complex rearrangements in the 4000-r pretreatment series, but still yields a χ^2 of 3.188 for one degree of freedom (P = ca. .068); this suggests that the distribution in the two series does not differ significantly. There is no satisfactory basis for comparing the frequencies with which rearrangements were induced in the 4000-r pretreated material and in BAUER's 4000- and 5000-r material, since the 4000-r treatment given by BAUER appeared to induce more breaks than were detected in our 4000-r controls. A comparison may be made, however, between the frequencies of the various types of rearrangements induced by BAUER's 4000-r and 5000-r treatments. When this is done, again grouping the data into two classes (two-, three-, four-break on one hand, and all the more complex on the other), a χ^2 of 4.604 is obtained (P = ca. .03), which indicates that the two series are significantly

different. This comparison, although not providing conclusive evidence, suggests that the increase in complexity of rearrangements that follows use of near infrared radiation in pretreatment may not keep pace with the coincident increase in frequency of the same rearrangements.

The positions of the induced breaks were plotted in these various experiments within the limits of the lettered subdivisions of the salivary-gland-chromosome maps, and it should be possible to determine whether or not the

TABLE 6

Comparison, using the χ^2 method, of frequencies of simple and complex rearrangements in experiments involving treatment with near infrared radiation followed by 4000 r of X-rays, with frequencies obtained with 4000 r alone.

TYPE OF TREATMENT	FREQUENCIES OF SIMPLE AND COMPLEX REARRANGEMENTS									
	2-BREAK		3-BREAK		4-BREAK		5-BREAK		MORE COMPLEX	
	OBS.	EXP.	OBS.	EXP.	OBS.	EXP.	OBS.	EXP.	OBS.	EXP.
24, 72 hr. I.R.+4000 r	128	(134.35)	28	(27.65)	33	(31.52)	11	(9.40)	9	(6.08)
4000 r controls	115	(108.65)	22	(22.36)	24	(25.48)	6	(7.60)	2	(4.92)

$\chi^2 = 4.581$ N=4 P=.337

increased frequency of chromosomal aberrations that follows the combination treatment is obtained by preferential breakage of specific chromosomes or regions thereof. Extensive data collected in earlier studies (KAUFMANN and DEMEREC 1937; BAUER, DEMEREC, and KAUFMANN 1938; BAUER 1939) had been interpreted as supporting the hypothesis that X-ray-induced breaks are distributed among the chromosomes in proportion to their length at late prophase or metaphase of mitosis; the percentages expected on this assumption are 22.7 in the X chromosome, 36.5 in the second, 38.8 in the third, and 2.0 in the fourth. Distribution of the 584 breaks obtained following exposure of males to either 24 hours or 72 hours of near infrared radiation and 4000-r of X-rays deviates somewhat from randomness, owing to an accumulation of breaks in the third chromosome and a deficit in the second chromosome. The χ^2 test (table 7) gives a probability (P) of about .03, which is slightly lower than the .05 generally considered requisite for confirmation of the hypothesis tested. By adding to the preceding the 266 breaks from the one-hour pretreatment experiments, a distribution more nearly at random obtains, the χ^2 test giving a value for P of about .07. Measured by the same test, the 257 breaks of the 4000-r controls gave a value of P of about .16.

Distribution of breaks in these two series of experiments may also be compared, inasmuch as the males were exposed simultaneously to the X-rays, to determine whether differences between them may be attributed solely to the sampling error. Such a comparison, using the χ^2 method, also gives a value of

P (about .05) that is at the threshold of significance (table 8). The controls used in this test provided only 257 breaks; if to these are added the breaks induced by 4000 roentgens in experiments conducted under parallel conditions, a group of 764 is available for comparison with the 584 (24-, 72-hour IR), or 850 (1-, 24-, 72-hour IR) breaks of the material receiving the combination treatments. Such differences as exist in break distribution between these groups may be attributed to sampling, since the tests afford values of P of about .12 and .18 (table 8). Much larger numbers of breaks are available for comparison if results obtained at dosage levels between 1000 and 5000 r are

TABLE 7

Comparison of observed distribution of breaks induced by 4000 r of X-rays after near infrared radiation, with values expected on the assumption that breaks are distributed at random on basis of chromosome length. 584 breaks from 24-hour+72-hour infrared series; 850 breaks from one-hour+24-hour+72-hour series.

	DISTRIBUTION OF BREAKS AMONG THE CHROMOSOMES				
	X	2	3	4	TOTAL
Observed	128	185	260	11	584
Expected	132.57	213.16	226.59	11.68	
	$\chi^2=8.844$	N=3	P=ca. .03		
Observed	179	288	368	15	850
Expected	192.95	310.25	329.80	17.00	
	$\chi^2=7.265$	N=3	P=ca. .07		

grouped; BAUER's summarized data (1939) include 2010. Their distribution among the chromosome limbs is not significantly different from that obtained when treatment with near infrared radiation precedes the use of X-rays (table 8). From all these considerations it appears, therefore, that the greater yield of chromosomal aberrations, as compared with the controls, that follows treatment with near infrared prior to X-radiation, is not due to selective sensitization of any one chromosome or chromosome limb.

Possible effects on specific regions within the chromosomes are more difficult to measure, because of the small number of breaks, the large number of divisions (102) among which they are distributed, and the disproportionately large number of breaks in chromocentral and intercalary heterochromatic regions. Measurements may be made, however, of frequencies recorded in the regions known to be essentially heterochromatic, such as those adjacent to the centromeres (20, 40, 41, 80, 81, 101), and 11A, 12DE, and 19E of the X. Of the 850 breaks observed in the material involving pretreatment, 183, or 21.53 percent, were in the proximal heterochromatic divisions, as compared with 59 of the total of 257, or 22.96 percent, in the 4000-r controls. Among the 2010 breaks recorded by BAUER, 404, or 20.10 percent, were located in these same regions. Thus the near infrared treatment does not increase the proportion of

X-ray-induced breaks in the proximal heterochromatic divisions that participate in the production of chromosomal aberrations. Nor do the intercalary heterochromatic regions seem to be differentially "sensitized," inasmuch as there were 81 breaks in divisions 11, 12, 19, and 20 of the X chromosome, or 9.54 percent of the total of 850 for the combination treatments, and 26 breaks,

TABLE 8

χ^2 tests to measure similarity of distribution of breaks induced by pretreatment plus 4000 r to distribution obtained in various other experiments.

TYPE OF TREATMENT	DISTRIBUTION OF BREAKS AMONG THE CHROMOSOMES								TOTAL
	X		2		3		4		
	OBS.	EXP.	OBS.	EXP.	OBS.	EXP.	OBS.	EXP.	
24, 72-hr. I.R. +4000 r	128	(135.41)	185	(191.65)	260	(243.04)	11	(13.89)	584
4000 r controls of same series	67	(59.59)	91	(84.35)	90	(106.96)	9	(6.11)	257
		χ^2=7.922		N=3		P=ca. .05			
24, 72-hr. I.R. +4000 r	128	(139.49)	185	(190.61)	260	(241.29)	11	(12.56)	584
4000 r (various exps.)	194	(182.51)	255	(249.39)	297	(315.71)	18	(16.44)	764
		χ^2=4.862		N=3		P=ca. .18			
1, 24, 72-hr. I.R.+4000 r	179	(196.42)	288	(285.94)	368	(350.19)	15	(17.38)	850
4000 r (various exps.)	194	(176.58)	255	(257.06)	297	(314.81)	18	(15.62)	764
		χ^2=5.899		N=3		P=ca. .12			
24, 72-hr. I.R. +4000 r	128	(138.66)	185	(198.31)	260	(235.90)	11	(11.03)	584
BAUER'S data (1000–5000 r doses)	488	(477.34)	696	(682.69)	788	(812.10)	38	(37.97)	2010
		χ^2=5.387		N=3		P=ca. .13			
1, 24, 72-hr. I.R.+4000 r	179	(198.23)	288	(292.44)	368	(343.56)	15	(15.75)	850
BAUER'S data (1000–5000 r doses)	488	(468.77)	696	(691.56)	788	(812.44)	38	(37.25)	2010
		χ^2=5.275		N=3		P=ca. .16			

or 10.2 percent of the total of 257, in the controls. From these limited data it appears that "sensitization" to X-ray breakage by near infrared radiation is not restricted to any specific chromosomes or portions thereof.

EFFECT OF PRETREATMENT ON DOMINANT LETHALS

As indicated previously, the breaks that are detected by analysis of salivary-gland chromosomes represent a residue of the larger number of potential breaks induced at the time of X-ray irradiation of the spermatozoa. Some of the potential breaks are presumably eliminated by restitution, which restores the original sequence of parts; other "potentially-broken" ends produce chromosomal derangements that furnish unbalanced, inviable nuclei in subsequent divisions. Since exposure of males to near infrared radiation prior to X-radiation increases the frequency with which the regions of potential breaks along the chromosome become available for participation in the formation of viable recombinations, it is also desirable to know whether such pretreatment increases the frequency with which such regions participate in forming the inviable or unbalanced types.

An effective measure of these dominant lethals can be had by determining the proportion of individuals that die in embryonic stages. Fatalities also occur during larval and pupal stages as a result of lethals induced by X-ray

TABLE 9

Proportion of eggs hatching among those deposited by females inseminated by males receiving type of treatment indicated, all other experimental conditions being uniform.

TYPE OF TREATMENT	TOTAL NO. EGGS DEPOSITED	NO. UNHATCHED 36 HRS. LATER	PERCENTAGE FAILING TO HATCH
None (controls)	7,047	478	6.8
Near infrared alone (48 hrs.)	14,272	1,901	13.3
2000 r X-rays alone	9,214	4,480	48.6
N. infrared (48 hrs.)+2000 r	9,562	5,096	53.3

treatment of the sperm, but as DEMEREC and FANO (1944) have shown, the proportions do not vary greatly within the dosage range between 1000 and 5000 roentgens. Accordingly counts were made of the numbers of eggs that hatched among the total deposited by females that had been inseminated by males exposed either to X-rays or to near infrared radiation plus X-rays. Preliminary counts showed that only about 20 percent of the eggs hatched following an X-ray dose of 4000 r, so that it seemed desirable to use a lower dose in order to determine the influence of the supplementary treatment. The results of the comparative study, using a 2000-r dose, are presented in table 9.

Treatment of spermatozoa with near infrared radiation prior to their exposure to 2000 r of X-rays afforded a slightly higher percentage of dominant lethals than that induced by 2000 r alone; but the difference between the two

percentages (53.3 and 48.6) is of the same order of magnitude as that between the two sets of "controls"—namely, the group of eggs fertilized by untreated sperm, and the group fertilized by sperm exposed only to the near infrared radiation. Evidently pretreatment of spermatozoa with near infrared radiation does not modify the frequency of dominant lethals to an extent comparable with the corresponding modification of the frequency of viable rearrangements. However, since the dominant lethals of the many-hit type, which more closely resemble in origin the viable rearrangements, constitute only a minor fraction of all dominant lethals, it is possible that the frequency of this small proportion is increased to a considerable extent by the pretreatment.

DISCUSSION

In the experiments here reported the frequency of chromosomal rearrangements induced by X-ray irradiation of the spermatozoa of Drosophila was modified either by pretreatment or by posttreatment with near infrared radiation. Posttreatment apparently accelerates those processes that make sperms that were not mature at the time of treatment available for transfer in copulation, and does not disrupt the chromosomes or modify their capacity for reattachment at the regions of potential breakage. The mode of action of near infrared radiation when used prior to X-ray treatment is not so clearly defined, although the data from a series of experiments reveal that pretreatment effects an increase in the frequency of viable types of rearrangement and perhaps also of multiple-break dominant lethals.

Might the augmented break frequency of the pretreatment series be attributable to some accelerating effect on biological processes similar to that produced by posttreatment? Such results as were obtained when near infrared radiation preceded the X-rays might be anticipated if among the fully-differentiated spermatozoa available for transfer in copulation there were cells that varied in their sensitivity to X-rays, the less mature being advanced during the pretreatment period to a stage in which their chromosomes were more susceptible to breakage by X-rays. Some data based on genetic techniques suggest the possibility of the existence of differential sensitivity of the chromosomes among batches of mature sperm. As a striking example, CATSCH and RADU (1943) reported that translocations between the second and third chromosomes of *D. melanogaster* were obtained at a significantly higher rate from spermatozoa transferred one day after irradiation than from sperm two to seven days after treatment. SHAPIRO (1931), although dealing with much smaller numbers, reported, on the contrary, a lower frequency of translocations in the one-to-six-day interval than in the six-to-16. In experiments bearing on this problem, most workers have grouped data for several days, so that differences between consecutive 24-hour periods are not indicated. In the experiments reported in the present paper, and in a number of similar studies during recent years, the males were mated with virgin females on the day of treatment, with other virgins on the third day, and occasionally also on the sixth day; in a few experiments females were provided at daily intervals. Except for the differences that may be attributed to sampling when groups are

of small size, the induced frequencies and complexities of rearrangements detected by analysis of salivary-gland chromosomes are quite similar among spermatozoa transferred during any portion of the twelve-to-fourteen-day period required for the cells that were immature at the time of treatment to become mature (the exception provided by posttreatment with near infrared radiation has previously been discussed).

If, as this evidence indicates, the spermatozoa available for transfer the first few days after X-ray treatment are a homogeneous lot, with respect to breakage of their chromosomes, the results obtained following pretreatment with near infrared radiation are not attributable to such factors as have been found to cause alterations in the frequency of derangement in experiments with other material (for example, differences in type of cell, age, genetic constitution, and phase of mitosis). Speed or freedom of movement of chromosomes is not involved, since recombination is apparently not effected during the entire period of treatment. It seems reasonably certain, moreover, that the near infrared radiation affects the chromosomes directly, rather than indirectly through interaction between extrachromosomal materials and products of the ionizing radiation at the time of treatment or later. Indirect action at the time of treatment is not in harmony with the generally accepted view that ionizing radiation acts directly on the chromosomes (for example, SAX 1942; LEA and CATCHESIDE 1942); and the possibility of delayed action is not supported by our finding that posttreatment is ineffective in modifying the recombination properties of chromosomes at regions of potential breakage. It should also be remembered that the sperm head, which contains the treated chromosomes, is essentially chromatic and colors uniformly following the Feulgen reaction.

Since the higher frequency of chromosomal aberrations induced by X-rays following pretreatment does not appear to depend on factors that influence the rate of such biological processes as cell division and spermiogenesis, the possibility of direct effects on the chromosomes must be considered. The greater frequency with which the viable types of rearrangements are detected following pretreatment indicates that a larger proportion of the spermatozoa utilized in fertilization, as compared with those receiving only 4000 r, carry the potential breaks capable of producing balanced combinations of chromosomes. This increase might be attained by modification or "sensitization" of the materials of the chromosomes (so as to increase the number or capacity for recombination of the regions of potential breakage induced by the X-rays), or by the utilization in recombination of breaks that give rise to dominant lethals when pretreatment is omitted. The latter alternative assumes that under certain conditions the thoroughgoing break that leads to the production of a dominant lethal is realized prior to the time that recombination occurs, but that pretreatment in some way inhibits the consummation of this process. Neither the studies of embryonic mortality reported in table 9, nor the knowledge that recombination to produce viable rearrangements occurs after insemination, lends any support to this possibility. It seems more reasonable, therefore, to assess the effect of near infrared in

terms of "sensitization" of the chromosome especially since the studies of hatchability of eggs do not eliminate the possibility that pretreatment increases the multiple-break type of dominant lethal as well as the viable type.

How might such "sensitization" be brought about? One possibility is a heat effect, since near infrared radiation raises the body temperature of Drosophila during the period of exposure. By means of a thermocouple designed by DR. J. GORDON CARLSON and the second author, it has been determined that the temperature of the fly during the period of exposure may be elevated as much as 7°C higher than room temperature. To what extent temperature contributes in "sensitizing" the chromosomes remains to be determined. In the experiments with unfiltered infrared radiation that provided a temperature of 39°C, some increase was obtained in frequency of rearrangements as compared with the controls. The fact that near infrared radiation produced comparable results at much lower temperatures within a similar period suggests that the over-all effect of temperature is not the primary factor involved, but that the radiant energy is absorbed by some component of the chromosome thereby inducing some loosening of the bonds that are subsequently uncoupled by the ionizing radiation, or causing physical changes in the chromosome. Energy in the infrared portion of the spectrum is absorbed and converted into rotation of molecules. Near infrared radiation of wave length 10,000 Å (the range used in the present experiments) provides about 1.2 electron volts of energy wherever a radiation quantum is absorbed. This amount might not be sufficient to produce a measurable effect on many kinds of molecules, but it is not inconceivable that it may cause certain types of chemical reaction. For example, little information is available concerning possible effects on the so-called macromolecules, especially the nucleoproteins which play such a dominant role in the functional organization of cellular materials. It may be such low-grade disturbances, unable in themselves to produce genetically detectable effects, that make the chromosomes more "sensitive" to X-rays and thereby increase the number of breaks available for participation in the production of viable recombinations. Further information may be gained by studies of the comparative effects of other wave lengths, or by extension of our knowledge of infrared absorption spectra.

Finally, we should like to point out that the experiments here reported, together with studies of interaction of X-rays and ultraviolet rays, offer substantial evidence that the capacity of X-ray-induced chromosomal breaks to participate in recombination is subject to modification. An extension of such experiments may offer clues for differential control of X-ray effects either in therapy or following accidental exposure.

SUMMARY

Modification of the frequency of X-ray-induced chromosomal rearrangements detected by analysis of the salivary-gland chromosomes of *Drosophila melanogaster* was effected by the use of near infrared radiation.

The supplementary treatment with wave lengths around 10,000 Å was obtained by utilizing the filtered radiation of an infrared source; the energy

so provided was sufficient to increase the temperature within the body of the fly as much as 7°C during treatment.

Posttreatment of males with near infrared accelerated the mitotic processes so that cells that were not mature sperm at the time of X-ray treatment became available for transfer in copulation several days sooner than if the males had been stored at temperatures within the range of 18 to 28°C. Posttreatment does not seem to be effective in eliminating the potential breaks induced by X-rays.

Pretreatment of males for 24 or 72 hours with near infrared radiation increases significantly the frequency of detectable X-ray-induced rearrangements as compared with the X-rayed controls. Increases were also obtained following one-hour treatments with either filtered or unfiltered radiation; the latter afforded a temperature of about 39°C.

The higher frequency of rearrangements obtained in the pretreatment experiments is not attributable to selective sensitization of any particular chromosomes or portions thereof.

Determination of hatchability of eggs deposited by females that had been inseminated by males exposed successively to near infrared and X-ray radiation suggests that pretreatment does not modify greatly the frequency of dominant lethals.

Various possible modes of action of the near infrared radiation in "sensitizing" the chromosomes to X-rays are considered.

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MODIFICATION OF THE FREQUENCY OF CHROMOSOMAL REARRANGEMENTS INDUCED BY X-RAYS IN DROSOPHILA. II. USE OF ULTRAVIOLET RADIATION

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CHROMOSOMAL rearrangements in Drosophila may be induced by X-ray treatment of the spermatozoa of the adult male. Although potential breaks are induced in the mature spermatozoon, new combinations among the chromosomes apparently do not arise until after the irradiated sperm has penetrated the egg in the process of fertilization (MULLER 1940; KAUFMANN 1940, 1941). Since the interval between irradiation and chromosome recombination can be extended in these flies over several days by withholding the males from copulation, an opportunity is offered to attempt to alter experimentally the capacity of the regions of potential breakage to recombine or to undergo restitution.

Following exposure to X-rays, chromosomes may be sensitive to types of treatment that leave no imprint on the genetic constitution of the normal cell. Supplementary energy necessary to affect the regions of potential breakage might conceivably be supplied either by chemical or by physical agents. In the first paper of this series we have described the effect on the frequency of chromosomal rearrangements of treating the spermatozoa of Drosophila with combinations of X-rays and near infrared radiation (KAUFMANN, HOLLAENDER, and GAY 1946). The present paper reports a set of experiments in which ultraviolet radiation of wave length 2537 Å was used following X-ray treatment. A preliminary report appeared in Year Book No. 41 (1942) of the CARNEGIE INSTITUTION OF WASHINGTON.

EXPERIMENTAL PROCEDURE

Males of the Oregon-R stock of *Drosophila melanogaster* were used within a few hours after emergence. The X-ray treatment of 4000 roentgens was given at the rate of 200 r per minute, using a Universal type Coolidge tube, with a tungsten target, operating at about 85 kilovolts and 5 milliamperes. Exposure to the ultraviolet radiation for a period of ten minutes followed shortly thereafter, except for those flies that were to be used to establish control cultures. Another set of controls was obtained from males treated only with the ultraviolet rays.

The ultraviolet radiation was delivered by a 15 watt low-pressure mercury vapor lamp with a glass envelope. About 80 percent of the total radiation emitted and 95 percent of that shorter than 3000 Å is wave length 2537 Å. The lamp was "burned in" for approximately 100 hours before using it ex-

perimentally in an effort to eliminate the wave lengths shorter than 2400 Å that might be emitted by a new lamp. Constant emission was assured by turning on the lamp at least 15 minutes before each treatment and stabilizing the voltage by means of a variac. To determine the amount of ultraviolet delivered by the lamp, a standardized thermopile with a quartz window was used. Very little infrared radiation is produced, and a single electric fan di-

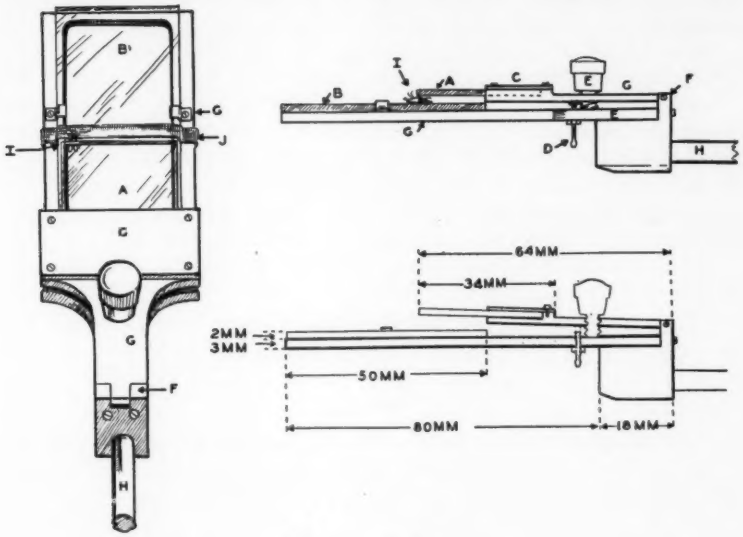

FIGURE 1.—Fly holder

A—Upper quartz plate.
B—Lower quartz plate.
C—Holder plate for upper quartz plate.
D—Pairs of screws for controlling distance between upper and lower quartz plates. Distance between plates about 0.15 mm.
E—Crude adjustment screw for controlling the distance between the two quartz plates.
F and G—Hinge and joint for holding upper quartz plate.
H—Arm for fastening fly holder to stand.
I—Fly in position to be pressed between quartz plates.
J—Cheese cloth holding fly in position under slight pressure against lower quartz plate.
(Holder built by L. R. CRISP, Natl. Inst. of Health).

rected to circulate air across the lamp was adequate to prevent overheating of the treated material. The flies were exposed to 1.8×10^5 ergs/cm^2.

Groups of ten males were treated simultaneously in a special holder (fig. 1). Following etherization, the abdomens of the flies were compressed between two quartz plates to a thickness of about .15 mm in order to bring the testes closer to the ventral body wall. Not more than 10 to 15 percent of the incident radiation of wave length 2537 Å will penetrate the chitinous exoskeleton (DURAND, HOLLAENDER, and HOULAHAN 1941), and additional absorption

occurs in the muscle and adipose tissues covering the testes. Variations in the thickness of these layers will influence the amount of radiation reaching the spermatozoa, as has been emphasized by MILLER (1941); MACKENZIE and MULLER (1940), and DEMEREC, HOLLAENDER, HOULAHAN, and BISHOP (1942). The theoretically ideal technique of collecting spermatozoa from females immediately after copulation and irradiating such material prior to artificial insemination of other females did not prove successful in a large enough number of trials to warrant its use as an experimental procedure.

Following the treatment, each group of ten males was placed in a culture bottle, together with ten to 12 virgin females of the same stock. Flies were transferred to new bottles at the end of three days. Two or three days later the males were discarded in order to ensure the use in fertilization of only those spermatozoa that were mature at the time of treatment. The effect of the treatment was determined by cytological analysis of chromosomal rearrangements found in the salivary glands of the third-instar female larvae. Aceto-orcein served to fix and stain the nuclei.

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RESULTS

The frequencies of chromosomal rearrangements determined in the various experiments are presented in table 1. No chromosomal aberrations were found among the 146 sperms tested from males that had been treated with only the

TABLE 1

Frequency of chromosomal rearrangements detected by analysis of salivary-gland chromosomes of progeny of males treated with X-rays and ultraviolet rays.

TREATMENT: X-RAY 4000 r-UNITS ULTRAVIOLET 2537 Å FOR 10 MINUTES	TOTAL SPERMS TESTED	FREQUENCY OF CHROMOSOMAL BREAKS					
		REARRANGEMENTS IN SPERMS TESTED		% SPERMS SHOWING REARRANGEMENTS	NO. OF BREAKS DETECTED	BREAKS PER 100 SPERMS	MEAN BREAK NUMBER
		ABSENT	PRESENT				
U.v. alone	146	—	—	—	—	—	—
X-ray alone	128	90	38	29.7±4.04	108	84.4	2.84
X-ray+u.v.	289	222	67	23.2±2.48	168	58.2	2.51

ultraviolet radiation, although it should be emphasized that deficiencies of one or two bands, such as SLIZYNSKI (1942) found associated with ultraviolet-induced lethals, could not be identified with certainty in the single pair of glands provided by each of the larvae selected. Since MULLER and MAC-

KENZIE (1939), MACKENZIE and MULLER (1940), and DEMEREC et al. (1942), using genetic methods of analysis, had likewise found that ultraviolet radiation does not produce gross chromosomal changes in Drosophila, the cytological analysis was not extended beyond the sample indicated in table 1. Likewise, the percentage of rearrangements among the X-ray controls was so similar to other values obtained after 4000 r treatment with the Cold Spring Harbor X-ray equipment, when the larvae were raised under comparable conditions, that extended analyses did not seem warranted. At the time that these experiments were completed, 549 pairs of glands had been examined following 4000-r dosages, and of these about 30 percent had been found to show chromosomal alterations, with 80 breaks per 100 sperms tested. It is thus evident that 4000 roentgens of X-rays produce in different samples of this material, under carefully controlled conditions, frequencies of chromosomal alterations that

TABLE 2

Frequency of rearrangements obtained following treatment with 4000 r+ultraviolet λ 2537 Å compared with frequency induced by 4000 r alone.

	REARRANGEMENTS AMONG SPERMS TESTED		
	NO. WITH ABERRATIONS	NO. WITHOUT ABERRATIONS	TOTAL
Observed	67	222	289
Expected	(81.40)	(207.63)	
Observed	169	380	549
Expected	(154.60)	(394.37)	
	$\chi^2=5.407$	N=1 P=*ca.* .02	

do not differ from each other in excess of the sampling errors. This uniformity provides a satisfactory basis for appraising the effects of supplementary treatment without resorting to the analysis of large numbers of pairs of glands.

When ultraviolet radiation followed the X-ray treatment, a marked reduction occurred in the frequency of rearrangements (table 1). Comparison of this frequency, by the χ^2 method, with that obtained following treatment of spermatozoa with only 4000 roentgens of X-rays, indicates that the differences are not referable solely to errors of sampling (table 2). Following the combination treatment the frequency of detectable chromosomal rearrangements was lowered from the level characteristic of 4000 roentgens to that of about 3000 r. For purposes of comparison, a treatment of 3000 r provided 184 pairs of glands from the F_1 female larvae, of which 43, or 23.4 ± 3.1 percent, showed chromosomal rearrangements, involving 96 breaks, or 52.2 per 100 sperms tested (the proportions of altered and unaltered types so closely resemble those obtained following the combination treatment that the χ^2 test gives a value of P between .95 and .98).

Considerable variability in the frequency of rearrangements was found

among the glands obtained from the many different cultures, each of which was secured by using a group of 10 males that had been exposed simultaneously to the ultraviolet radiation. Since the number of larvae per culture was small, the differences between these samples were not quite statistically significant (the χ^2 test for homogeneity gives a value of P of about .23), although in some of the experiments the frequency of rearrangements was reduced to about 15 percent, whereas in others it remained at about the 30 percent level characteristic of the X-rayed controls. Such differences may be attributable in part to the degree of penetration of the ultraviolet rays into the tissues of the fly. Variable factors among individuals would be the amount of flattening, and the thickness of the cuticula, muscle-fiber layer, and adipose tissue covering the testes. Moreover, as MILLER (1941) has shown, the bases of the testes and seminal vesicles, in which the mature spermatozoa accumulate, are frequently covered by other portions of the testes. Recovery of the flies from the operation of flattening the abdomen is not always complete; the threshold between the degree of pressure necessary to secure penetration of the ultraviolet to the testes and that resulting in death or in sterility is not easily determined, and there is a tendency to err on the side of reduced rather than of exaggerated pressure. For these reasons the total data for the combined treatments must include an indeterminable number of pairs of glands whose set of paternally derived chromosomes came from spermatozoa that were affected by the ionizing radiation of the X-rays but were not reached by the ultraviolet quanta.

EFFECTS OF RADIATION ON EMBRYONIC MORTALITY

The reduction in the frequency of detectable chromosomal rearrangements effected by posttreatment of X-rayed sperm of Drosophila is probably to be attributed to modification of the capacity of regions of potential breakage to form viable new combinations. If the ultraviolet radiation should alter the chromosomes at regions of potential breakage, so as to inhibit restitution and recombination, the zygotes arising from eggs fertilized by spermatozoa containing such broken chromosomes should not proceed far in their embryonic development. If, on the other hand, ultraviolet radiation should increase the amount of restitution, a larger proportion of spermatozoa should transmit a normal complement of chromosomes in fertilizing the eggs. The method of operation indicated in the first of these alternatives, therefore, should increase the frequency of dominant lethals, whereas the method indicated in the second alternative should decrease such frequency. Counts were made, accordingly, of the numbers of eggs that hatched when fertilized by spermatozoa that received both X-rays and ultraviolet rays, for comparison with the controls. Inasmuch as the 4000 r dosage level produces about 75 to 80 percent of lethals that operate in embryonic stages, it seemed desirable in measuring hatchability of eggs to reduce the X-ray treatment to 2000 r, so that possible effects of the combination treatment might be appraised more accurately. The results are presented in table 3.

About 7 percent of the eggs failed to hatch among the untreated controls, and about 19 percent in the ultraviolet-treatment series. The difference in per-

centages is 3.9 times its standard error computed on the basis of dispersion of partial samples, and therefore referable to factors other than variability within the two series. Since parallel culture conditions were provided in both sets of experiments, the higher percentage of embryonic mortality obtained following treatment with ultraviolet radiation is attributable either to induced sterility or to dominant lethal changes. As reported by MACKENZIE and MULLER (1940), and by DEMEREC et al. (1942), males of Drosophila may be sterilized

TABLE 3

Hatchability of eggs deposited by females inseminated by males receiving type of treatment indicated, all other experimental conditions being uniform.

TYPE OF TREATMENT	TOTAL NO. OF EGGS DEPOSITED	NO. UNHATCHED 36 HRS. LATER	PERCENTAGE FAILING TO HATCH	
			MEAN	STANDARD DEVIATION OF PARTIAL SAMPLES OF *ca.* 500
None (controls)	7,047	478	6.90±0.74	2.47
Ultraviolet λ 2537 Å	13,974	2,523	18.94±3.03	15.72
2000 r	9,214	4,480	48.31±1.21	4.69
2000 r+u.v.	9,418	5,119	54.16±3.07	13.37
Controls *vs.* u.v. alone			12.04/3.12=3.9	
2000 r+u.v. *vs.* 2000 r alone			5.85/3.30=1.8	

by treatment with ultraviolet radiation. It is also known that virgin females, or those failing to receive sperm during copulation, deposit eggs that do not hatch (KAUFMANN and DEMEREC 1942). To guard against the inclusion of large numbers of such unfertilized eggs, counts were restricted to the first few days of laying, and cultures were excluded in which none of the eggs produced viable larvae. Under such conditions practically all of the females tested had been inseminated, and fertilization was probably effected in nearly all of the eggs deposited, since polyspermy prevails in Drosophila. Failure of the eggs to hatch may be referable, therefore, to chromosomal derangements effecting embryonic mortality. Inasmuch as chromosomal rearrangements depending on two or more breaks are not produced by ultraviolet radiation with any appreciable frequency in Drosophila, the dominant lethals are probably of the single-break type, such as constitute the major portion of the X-ray-induced lethals that act in embryonic stages (PONTECORVO 1942; DEMEREC and FANO 1944). That ultraviolet radiation can induce chromosomal breaks in Drosophila has been shown by SLIZYNSKI (1942). SWANSON (1942) has also reported that wave length 2537 Å induces chromatid breaks in the pollen tubes of Tradescantia.

When exposure to the ultraviolet rays followed the X-ray treatment, there was no significant increase in the frequency of dominant lethals as compared with the X-ray controls. The 54 percent obtained following the combined treatment is close to the value expected on the basis of the cumulative effect

of the 19 percent of dominant lethals provided by the ultraviolet rays and the 48 percent induced by 2000 r of X-rays (48 plus 19, less 9 percent affected by both types of radiation). Since these data indicate that the single-break type of chromosomal aberration is not increased to any appreciable extent by the action of ultraviolet radiation on the X-ray-induced potential breaks, the effect of posttreatment in reducing the frequency of chromosomal rearrangements appears to be due to increased restitution. SWANSON's (1944) data on Tradescantia also suggest that ultraviolet radiation of wave length 2537 Å increases restitution among breaks previously induced by X-rays.

DISCUSSION

Ultraviolet radiation of wave length 2537 Å that penetrates the mature spermatozoa of Drosophila following their exposure to X-rays is effective in reducing the frequency, as compared with the controls, of chromosomal rearrangements detected by analysis of salivary-gland chromosomes. It is known that ultraviolet radiation is not absorbed equally by all cellular components (CASPERSSON 1940). Wave length 2537 Å is absorbed by the nucleic acids, but it remains to be determined whether the action here reported is attributable to absorption by ribose nucleic acid, or by desoxyribose nucleic acid or other components of the nucleus. SWANSON (1942, 1944) attributed the inhibiting effect on X-ray-induced breaks in the pollen tubes of *Tradescantia paludosa* to changes in the matrix, since the chromosomes were markedly shortened after exposure to wave length 2537 Å (not after treatment with 2967 Å or 3022 Å). Such details of chromosome morphology are not discernible in the sperm nucleus of Drosophila, but since it colors uniformly following application of the Feulgen technique and is not digested by ribonuclease (SCHULTZ 1941), it appears that the nucleic acid is chiefly of the desoxyribose type. Additional studies are required to determine the effects on X-ray-induced breaks of monochromatic radiation of wave lengths that are absorbed primarily by other components of the chromosome.

The sequence of events that follows absorption and leads to the inhibition of chromosome recombination also remains unknown. In the absence of direct evidence some suggestions concerning possible modes of action are provided by studies of the sensitivity of nucleic acids and proteins *in vitro*. Limited amounts of 2537 Å will readily depolymerize a highly polymerized aqueous solution of sodium thymonucleate (HOLLAENDER, GREENSTEIN and JENRETTE 1941). Ribose nucleic acid, which shows practically no polymerization in aqueous solution, is not degraded by small amounts of 2537 Å. It is only when high intensities of 2537 Å are used that the chemical structure of ribose as well as desoxyribose nucleic acid is broken down. The energy values required for such changes exceed those used in our experiments. When serum proteins are treated *in vitro*, radiation of wave length 2537Å effects an increase in viscosity, a decrease of the colloid osmotic pressure, and a homogenizing of the electrophoretic pattern. Apparently unfolding of the protein occurs first, then splitting of the unfolded molecules, and finally aggregation (DAVIS, HOLLAENDER, and GREENSTEIN 1942). If proteins of comparable structure and be-

havior exist within the chromosomes of the spermatozoa of Drosophila, it is possible that the energy absorbed following the use of 2537 Å may initiate the first step of this sequence. Unfolding of the protein, combined with depolymerization of the thymonucleic acid, may possibly so alter the chromosomes at the positions of the potential breaks that their capacity for recombination is decreased.

SUMMARY

Ultraviolet radiation of wave length 2537 Å used subsequent to X-rays in the treatment of the spermatozoa of *Drosophila melanogaster* effected a decrease, as compared with the controls receiving only an equivalent dose of X-rays, in the frequency of chromosomal rearrangements.

In order to treat the male flies with the ultraviolet radiation their abdomens were flattened between the quartz plates of a holder designed for this purpose.

About 19 percent of the eggs failed to hatch that were deposited by females that had been inseminated by males treated with λ 2537 Å, as compared with 7 percent in the untreated controls. The difference between the two groups is attributed to induction by the ultraviolet radiation of the single-break type of dominant lethal.

Other studies of hatchability of eggs deposited by females that had been inseminated by males treated with either 2000 r of X-rays, or 2000 r plus λ 2537 Å suggest that the effect of posttreatment in reducing the frequency of chromosomal rearrangements is attributable to increased restitution rather than to an increase in the frequency of the single-break type of chromosomal derangement.

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SPONTANEOUS MUTATION AT THE R LOCUS IN MAIZE. I. THE ALEURONE-COLOR AND PLANT-COLOR EFFECTS

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THE study of spontaneous mutation is laborious at best, but it seems an indispensable preliminary to the interpretation of mutations induced experimentally, and it may be the only approach now open for the study of gene evolution. Genetic studies with X-rays were begun chiefly in order to make possible the experimental analysis of gene mutation. While these studies have greatly advanced our knowledge of the chromosomal mechanism, they have added little to our knowledge of the gene. Their chief value in this connection has been in showing clearly that mendelizing variations may be produced by various types of chromosomal alteration, and in thus emphasizing the need for criteria distinguishing the occurrence of new gene forms from the occurrence of changes due to loss, duplication, or rearrangement of genes. In view of the known phenotypic effects of various chromosomal derangements produced by X-ray treatment, it is doubtful that any of the evidence on induced gene mutation provides valid proof of an effect of X-rays upon the frequency of occurrence of new gene forms. The apparent mutations induced by ultraviolet radiations are less dubious (STADLER 1941), but these also are in need of further critical study.

Such criteria may be developed only by the detailed study of mutational phenomena at specific loci. These loci may then be used in analyzing the effects of apparent mutation-inducing agents, and in the study of other problems of gene mutation. This necessity for focusing the study upon the individual gene of course intensifies the technical difficulties arising from the rarity of spontaneous mutation.

It is feasible in maize, because of certain technical advantages, to identify mutations in very large populations. It is thus possible to assemble spontaneous mutations of specific genes in sufficient number to permit comparison with groups of mutants of the same genes induced by various treatments, and in some cases to permit quantitative study of certain problems of spontaneous mutation. The method may be used to identify recessive mutations of genes affecting endosperm characters or, with some additional labor, genes affecting seedling characters. It is most effectively used with mutants occurring in female gametes, but with added tests to exclude the possibility of error from pollen contamination it may be used with mutants occurring in male gametes.

The gene *R* was originally identified as a dominant gene for red or purple color in the aleurone layer of the endosperm (EAST and HAYES 1911). EMERSON

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(1921) showed that this gene is involved also in the determination of red and purple plant color, and that both colored aleurone and colorless aleurone alleles occur with both "colored" (that is, red or purple) and "colorless" (that is, green) plant effect. The correlation of seed and plant color which occurs in hybrid progenies of certain combinations among these alleles had been noted by WEBBER (1906). EMERSON distinguished the four major types as follows:

R^r Colored aleurone, colored plant
R^g Colored aleurone, colorless plant
r^r Colorless aleurone, colored plant
r^g Colorless aleurone, colorless plant

If crossing over ever occurs between the determiners of aleurone and plant color, it must be exceedingly rare. EMERSON (personal communication) tested several thousand seedlings from the cross $r^g \times R^g/r^r$. In general, the colored seeds gave colorless seedlings, and the colorless seeds colored seedlings, but there were rare exceptions. These were confirmed as true exceptions by digging up the seedlings to confirm the seed color classification. Several of these exceptions were grown to maturity and selfed, to determine the seed color genotype of the plant directly, rather than by inference from the color of the endosperm of the seed planted. In each of these cases it was found that the seed color of the plant was as expected with no crossing over—in other words, the supposed exception represented a seed which had one allele of R in its endosperm and the contrasted allele in its embryo. It was later shown by SPRAGUE (1929, 1932) that "hetero-fertilization" (fusion of egg and polar nuclei with sperms of unlike genotype) is not rare in maize. The exceptions tested may be accounted for by this mechanism, and the total of exceptions observed is not greater than might be expected from this cause. It may be noted also that mutation of both R^g and r^r to r^g occurs with appreciable frequency in various races of maize. Such mutants could be distinguished from crossovers only by additional controls. No experimental data are now available to indicate whether or not crossing over may occur with extremely low frequency, but it is evident that if two separable factors are involved, the linkage must be extremely close.

Several additional alleles, distinguished by various degrees of intensity or extension of aleurone color or of plant color have been described (summary in EMERSON, BEADLE, and FRASER 1935). There is evidence that the R^r and r^r alleles found in different cultivated races represent many distinguishable levels of plant color expression (STADLER 1942; STADLER and FOGEL 1943). The four types listed above are to be regarded therefore not as single alleles, but as groups of alleles, the alleles within the groups being characterized by distinct levels of expression in aleurone color and in plant color. These alleles constitute a non-linear series, in which apparently any of a wide variety of levels of plant-color effect may occur with or without aleurone-color effect. Conversely, distinct levels of aleurone-color effect occur among both colored plant and colorless (or nearly colorless) plant alleles. In all cases tested, the presence of color in any tissue is dominant to its absence, and stronger pigmentation is dominant to weaker.

Spontaneous mutation of alleles of the R^r group affects aleurone-color expression and plant-color expression independently (STADLER 1942). Thus R^r mutates regularly both to r^r and to R^g, but not to r^g. This is as would be expected if the R^r effect is due to the action of two closely linked but independent genes, one affecting aleurone color and the other affecting plant color. The present paper is concerned with the further study of the relation between the aleurone- and plant-color effects, as determined from spontaneous mutations of various R^r alleles.

TECHNIQUE FOR IDENTIFICATION OF SPONTANEOUS MUTATIONS IN MAIZE

Any method used in identifying spontaneous mutations for significant comparisons of mutation frequency must permit the testing of extremely large populations of gametes. In addition, it must eliminate the possibility of occurrence of the mutant genotype by any kind of uncontrolled mating, since no precautions against contamination can insure against its possible occurrence in such rare instances as would suffice to vitiate the mutation evidence. Finally, the method should permit the recognition of any irregular segregation or chromosomal derangement which might give results simulating mutation in the mating system used.

The chief advantages of maize for this purpose are the following:

(1) An abundance of endosperm characters determined by known genes of regular inheritance. These include several affecting pigmentation (*A*, *A2*, *C*, *R*, *Pr*, *In*, *Y*), and several affecting endosperm composition (*Su*, *Wx*, *Sh*, *Bt*). Each of the genes listed is distinguished from a recessive allele by a conspicuous phenotypic effect, so that on inspection of the seeds of an ear heterozygous for any of these genes, the occurrence of a single seed of the recessive phenotype is unmistakable.

(2) The possibility of controlled crossing *en masse* by detasseling the female parental stock in an isolated crossing field. In practice the multiple recessive pollen stock is planted in every fourth row, the intervening rows being planted to the various multiple dominant stocks in which mutation frequency is to be determined for the female gametes. All plants of the female parent stocks are detasseled before pollen shedding occurs, so that the only pollen available is that from plants of the pollen stock. Since no hand pollination is necessary, virtually unlimited populations may be produced, and the factor limiting the populations used is the quantity of ears which may be examined. With this method of mass crossing, pollen contamination is not absolutely excluded, but its extent as shown by the frequency of seeds of dominant phenotype on ears of the multiple recessive stock is a small fraction of 1 percent.

(3) The regular seed set of the maize ear, which makes lethal segregations obvious.

(4) The sensitivity of the male gametophyte in the detection of developmental defects which may be associated with the mutant allele in haploid tissue. If a plant heterozygous for the mutant allele transmits the mutant in normal ratio through pollen, the physiological effects of the mutant in haplo-

phase must be so nearly normal as to give no significant advantage or disadvantage in competitive pollen tube growth.

Several years ago, over a period of years, data were collected to indicate the frequency of spontaneous mutation for eight of the maize genes affecting endosperm characters. The results have been reported elsewhere (STADLER 1942).

The method used in the identification of spontaneous mutations among female gametes may be described as follows, using as an example the mutation of R^r to a colorless aleurone allele:

Stocks homozygous for R^r R^r (and for various other dominant genes with endosperm effects) are pollinated *en masse* by a stock homozygous for the recessive r^0 r^0 (with the additional homozygous recessive genes). The seeds produced by this cross are colored, like the female parent, with an occasional colorless seed presumably due to mutation of R to r. The presumed mutants are planted and are backcrossed by the multiple recessive pollen parent stock. The production of a colorless ear with full seed-set from this pollination confirms the mutation. In some cases the mutant is selfed, and the backcross test is made on ears of the recessive stock. Certain additional tests concerned with the nature of the mutants have been applied in a large number of cases but are not maintained as routine confirmatory tests. These will be described presently. Consider first the alternative explanations of the absence of aleurone color in the presumed mutant seed:

(1) Non-genetic causes. This possibility is excluded by the progeny test.

(2) A genetic alteration affecting the endosperm and not the embyro, and thus not subject to further testing to distinguish other genetic alterations from mutation. This is excluded by the backcross test, since this is a test of the genotypic constitution of the embryo of the seed which was identified as a mutation by the character of its endosperm.

(3) Pollen contamination. Pollen contamination could not make the seed colorless through lack of R, since R in this mating comes from the female parent. Pollen contamination introducing a dominant inhibitor of aleurone color, X, could produce a colorless seed $R\ r\ X\ x$, but this when backcrossed to the $r\ r\ x\ x$ parent stock would give 25 percent colored seeds. With linkage between R and x, the proportion of colored seeds on the backcross ear would be between 25 percent and 50 percent.

Contamination by a recessive inhibitor (in other words, the recessive allele of a dominant color gene complementary to R) could not produce a colorless grain unless the female parent carried the same recessive. This requires that we assume a mutation at the inhibitor locus, since the homozygous colored stock from which this plant was grown could not have bred true for color if it had carried the recessive inhibitor. Assuming the presence of a recessive inhibitor z in the female gamete, and the introduction of the same recessive by pollen contamination, the colorless grain produced, $R\ r\ z\ z$, would give 50 percent colored progeny on backcrossing to the r tester, $r\ r\ Z\ Z$.

(4) Exceptional segregation from a relatively true breeding heterozygous stock carrying the r gene, if it were by some means almost completely prevented from producing functional r gametes, might be mistaken for mutation

to *r* in a homozygous *R R* stock. The mechanism of balanced lethals, or other conditions eliminating the recessive segregates by linked lethal effects, could not cause this difficulty in maize, since the absence of 50 per cent of the seeds would be clearly evident on the ears examined for mutations. The mutants occur commonly as single colorless seeds on full-set ears. Large sections of these ears, with perfect set, show that *R* was present in the embryo-sac genotype in the ovules in general, not merely in a surviving fraction of them.

Another mechanism which might conceivably result in the segregation of *r*-gametes in extreme low frequency is the presence of numerous duplications of *R* with inheritance sufficiently irregular to permit occasional production of a megaspore without duplication. This is extremely improbable, but the "B chromosomes" found in certain races of maize (RANDOLPH 1928, 1941) are inherited in a manner which could make it possible. The B chromosomes are relatively inert genetically, and may be present in numbers ranging to 20 or more without observable phenotypic effect. Now if these chromosomes carry an *R* allele, or have an effect similar to it, a plant heterozygous or homozygous for *r* in its euchromosome genotype would yield gametes of *r* effect only in those cases in which a megaspore failed to receive any of the B chromosomes. This could be low enough in frequency to give the appearance of a true breeding *R R* race, with rare mutations to *r* and with no associated sterility. In the earlier experiments on spontaneous mutation, two or more sibs of each presumed mutant were selfed to exclude such possibilities of mutation simulated by recessive characters segregating with very low frequency. This test was applied in the case of many mutations of *R* and of the other genes affecting endosperm characters. The unmutated sib plants invariably gave regular one-factor ratios for the genes in question, showing that the plants which yielded the mutants were simple heterozygotes for the genes involved. Routine testing of this point was therefore discontinued. RANDOLPH (1941) has shown that the presence of B chromosomes is without effect upon the segregation ratios for 53 genes well distributed over the chromosomes of maize.

(5) Androgenesis. In the progeny of the cross *R R*×*r r*, a colorless seed could possibly be produced by the development of the endosperm, or possibly both embryo and endosperm, from the sperm nuclei, without fusion with the female germ cell nuclei. This phenomenon has never been reported in maize, but COLLINS and KEMPTON (1916) found wholly paternal inheritance in an intergeneric cross of two related grasses, *Tripsacum dactyloides* L.× *Euchlaena Mexicana* Schrad.

In the case of the mutants of R^r it is evident that androgenesis is not involved, since the mutant allele, r^r, is distinguishable from the allele in the pollen parent, r^g. This argument does not apply in the case of the other endosperm characters, however, since the mutant alleles identified are in most cases indistinguishable from those of the male parent stock. But if androgenesis were the cause of the occurrence of seeds of the recessive phenotype, the multiple recessives of the pollen stock would occur together in each of the mutant seeds. No such seeds have been found on any of the ears tested for mutation.

(6) Segmental deficiency. As a general criterion in the detection of the

results of deficiency, the development and functioning of the male and female gametophyte is probably more sensitive than direct cytological examination. This is certainly not true for every chromosome region considered individually, for McCLINTOCK (1944) has demonstrated cytologically the occurrence of minute terminal deficiencies of chromosome 9 with normal transmission through both male and female germ cells. The deficiencies in these cases were detectable through reduction in the size of the last chromomere, or in some cases only through the absence of a portion of the thin strand which joins this chromomere to the terminal heterochromatic knob. It is clear that neither the absence of cytologically observable deficiency nor the absence of a detectable effect upon gametophyte development is valid disproof of the possibility of minute deficiency.

There is in general, however, a close relation between deficiency and defective gametophyte development. Deficiencies extensive enough for cytological identification are in the main lethal to both the male and female gametophyte, and are manifested in the heterozygous plant by the abortion of half of the pollen and half of the ovules. Instances are not infrequent of cytologically obvious deficiencies which are haplo-viable. In some cases ovule fertility is much reduced, in some fertility is normal but the heterozygous-deficient seeds produced by the deficient female gametophytes are reduced in size, and in a few cases a full set of fully developed seeds is produced. Similarly the deficient pollen grains, in various known cases of cytologically detectable deficiency, range through various levels of visible defectiveness, and a few cases are known in which the deficient pollen is wholly normal in appearance, though non-functional in competition with the non-deficient pollen produced by the heterozygous deficient plant.

The relation between deficiency and the genetic criteria applicable to the gametophyte may be illustrated by certain X-ray-induced deficiencies at the *A* locus (STADLER and ROMAN 1943). The deficiency designated *a*-X3 is transmissible through the female gametophyte, though with much lowered frequency and with the production of distinctly small seeds. Pollen with *a*-X3 is reduced in size and in starch content, and is non-functional. The deficiency is not visible cytologically, though since the chromosome region concerned is relatively unfavorable for cytological examination, this does not indicate that there may not be cytological deficiency of the order demonstrated in the minute terminal deficiencies of chromosome 9. The case is identified as a minute segmental deficiency by genetic evidence, for in *a*-X3/*a*-X3 plants carrying an unstable duplication which covers the *A* region, sectors resulting from somatic alteration and loss of the duplication show that other genes as well as *A* are included in the segment affected. Two additional deficiencies in the same region, *a*-X2 and *a*-X1, were also induced by X-ray treatment. These give similar genetic evidence of deficiency, but are much less extreme in their effects upon gametophyte development. Deficiency *a*-X2 is transmissible through female germ cells, with a moderate reduction in ratio and with no appreciable effect upon size of seed. It produces pollen which is normal in appearance and which is functional, though in greatly reduced ratio, in competition with non-deficient

pollen. Deficiency *a*-X1 shows little if any reduction in female transmission. It produces pollen of normal appearance which is functional in distinctly higher proportion than that with *a*-X2, though in lower proportion than normal. The three cases presumably represent various grades of deficiency, which are close to if not beyond the limit of cytological detection, and which are characterized in varying degree by reduced transmission through the male gametophyte.

The spontaneous mutations have therefore been examined in all cases for visible pollen defects and have been tested in a large number of cases for evidence of reduced transmission through pollen. The latter test is easily made in the case of the mutants from R^r, since the original mutant plant is heterozygous $r^r r^g$, and pollen transmission of the mutant allele r^r is shown by a cross on the tester stock $r^g\ r^g$. In addition, in many instances, the mutant plant is crossed with an *R* stock to produce *R* r^r plants, which permit transmission tests more easily by the use of seed color instead of seedling color classification of the progeny. In the case of genes other than *R* this indirect method is necessary to bring the mutant gene into combination with a distinguishable allele. These tests give no indication whatever of a gametophytic viability effect associated with the spontaneous mutations.

This result shows that segmental deficiency of irregular extent is not the cause of the spontaneous mutants. Although presumed mutants due to segmental deficiency would not invariably be detectable by reduced pollen transmission, a population of presumed mutants due to deficiency would certainly include very frequent cases with detectable pollen effects.

It shows also, for the specific genes concerned, that spontaneous mutation has no necessary connection with viability effects, even when viability is tested by the very rigorous standard of competitive functioning of the haploid gametophyte. In this respect, the spontaneous mutants as a group contrast sharply with the mutants obtained from X-ray-treated material, among which a large proportion show associated viability effects.

(7) Single-gene deficiency. A possible mechanism of gene mutation is failure of reproduction of the gene concerned. The resulting single gene deficiency could not be expected to be cytologically detectable, and there is no basis for assuming that it would necessarily have any viability effect.

If R^r is considered a single gene, and if the gene is assumed to be the unit of molecular reduplication within the chromosome, this mechanism is excluded in the case of the spontaneous mutations of R^r. Spontaneous mutation of R^r yields intermediate alleles of positive effect (r^r or R^g), and only very rarely the "null" allele phenotypically equivalent to R^r-deficiency (r^g).

If R^r is considered to represent two closely linked genes, one affecting aleurone color and the other plant color, this argument does not hold, but a similar argument may be advanced at least for the mutations affecting the aleurone-color gene assumed. Spontaneous mutations of aleurone color to intermediate levels have been identified in several instances in the case of R^r alleles which have also yielded mutations to colorless aleurone. The frequency of mutation to intermediate levels has not been determined, since it is not possible to make

an objective identification of mutants at all possible intermediate levels. Mutation frequency determinations are therefore made on the basis of colorless mutations only. In the course of these examinations several instances of near-colorless seeds have been identified, and have been confirmed as mutations of *R* to intermediate levels of aleurone-color expression. These will be described in a later paper of this series.

The assumption that the gene, as experimentally identified, is the unit of molecular reduplication is of course to be regarded as no more than a tentative hypothesis. It is possible that the portion of the gene string which is identified by genetic analysis as a single gene locus is in fact a complex of several independently reduplicating elements. Failure of reduplication of individual elements might reduce the total effect of the complex to various levels recognizable as intermediate alleles of distinctive effect. In other words, the possibility of allelic differentiation by presence or absence of postulated sub-genes is one which cannot be excluded by experimental design; it is rather a hypothesis of gene structure which can be compared with others in the interpretation of the detailed experimental results. The chief purpose of experiments of the kind here described is to secure detailed evidence on the mutational behavior of specific genes in order to provide a basis for the evaluation of such hypotheses.

(8) Unequal crossingover. The gene *R* is located on the longer arm of chromosome 10 and is apparently very near the end of the chromosome. There is no gene beyond *R* on the linkage map. A terminal knob on the longer arm of chromosome 10, which was found by LONGLEY (1938) to occur in certain stocks, was reported by RHOADES (1942) to give 1–2 percent crossing over with *R* in plants in which crossing over in the adjacent segment *R-g* was apparently normal. The lack of suitable marker genes has made it impossible to determine the possible relation of crossing over to mutation of *R*. This should now be possible by the use of the terminal knob as a distal marker, and stocks are being prepared for this purpose.

OCCURRENCE OF ALEURONE- AND PLANT-COLOR MUTATIONS IN THE INDIVIDUAL PLANT

In order to secure recurrent mutations of both types from the same plant, mutations in male gametes were identified. This makes possible the production of large progenies from gametes of the single plant, but it makes necessary additional precautions to exclude the possibility of pollen contamination. Two sib plants of homozygous R^r: Columbia (an allele extracted from a genetic tester stock) were crossed upon numerous ears of an r^g stock. The male parent was homozygous for the marker genes *Pr Sh Wx Su Pl j*, all of which were present as contrasted alleles in the female parent stock.

Mutations affecting aleurone color were identified by inspection of all the seeds on the ears, and those affecting plant color by inspection of seedlings grown from the total population except for small remnant reserves for each ear.

The examination for aleurone-color mutation is simple and precise in tests

of mutation in female gametes, since endosperms of genotype *R R r* are fully colored and the colorless seeds from mutation are unmistakable. In tests of male gametes the examination for mutant seeds is less precise, for the non-mutant seeds, of genotype *R r r*, are mottled rather than full-colored. A combination of stocks giving rather heavily mottled *R r r* seeds was used for the experiment, and all ears in which mottling was too light to permit definite identification of *r r r* seeds were discarded. All colorless seeds were tested as mutations except those identified by the presence of *su* and *sh* as due to self-contamination of the female parent stock.

The examination for plant-color mutations is made in seedling flats by discarding all seedlings showing anthocyanin pigmentation in the seedling leaf-tip, and transplanting for further examination and test all those without leaf-tip color. R^r: Columbia, and all other R^r alleles used in the studies reported in this paper, give distinct anthocyanin pigmentation in the seedling, clearly evident in the mesocotyl, the coleoptile, and the seedling leaf tip. Seedling leaf-tip color is evident in each leaf almost immediately on emergence, while coleoptile and mesocotyl color develop more slowly and with more irregularity in level of early expression. Tip color provides a basis for wholly objective classification of the seedlings, which may be applied successively to each emerging leaf. The procedure adopted for identification of plant color mutations is to discard immediately all seedlings showing positive leaf-tip color, retaining the others for further examination up to the four-leaf stage. All seedlings not showing leaf-tip color at this stage or earlier are tested as presumed plant-color mutations. Several R^r alleles of weak plant color expression are known, in which the mesocotyl and coleoptile are colored but in which no color is evident in the leaf tip. Mutation to these intermediate levels, as well as mutation to the colorless plant type, would be identified by loss of seedling leaf-tip color.

The tests of both aleurone- and plant-color mutations included selfing of the mutant plant and crossing to the tester *A C* R^g/r^{ch} *j pl*. The characters of the mutant seeds and plants, and of the seeds and plants produced by these pollinations, show the genotype of the mutant as to *R* and all of the marker genes. The establishment of the genotype as r^r/r^g, in the aleurone mutants, or R^g/r^g in the plant-color mutants, confirms the mutation and shows that the aleurone- and plant-color action of the parent gene were separately affected. The establishment of the genotype for the marker genes shows that the substitution of a different allele in the presumed mutant individual was not due to pollen contamination.

In a few instances mutant plants were not sufficiently tested to establish these points, and these are separately listed as possible additional mutants not fully tested. With the exception of these plants, all members of the population of seeds examined may be classified definitely as mutant or non-mutant for loss of aleurone color, and all members of the population of seedlings examined may be similarly classified for mutation affecting plant color. As regards mutation for loss of both aleurone color and plant color, the entire

population of seeds examined may be definitely classed as non-mutant, with the exception of the two colorless seeds which failed to germinate. The data are summarized in table 1.

The frequency of aleurone-color mutants and that of plant-color mutants are of the same order. The former were about twice as frequent in the populations tested, but the difference is not clearly significant.

As noted above, the criterion of plant-color mutation was such as to permit the identification of mutations to intermediate levels of plant-color expression as well as to the null level. In a sample of 22 R^r alleles studied by STADLER and FOGEL (1943, 1945) there were included several R^r's of relatively weak plant-color expression. None of these show seedling-tip color, but all show clear coleoptile color, and all but one clear mesocotyl color. These alleles in general have very little plant color at the flowering stage, and some plants are indis-

TABLE 1

Aleurone- and plant-color mutations of R^r:Columbia in individual plants.

PLANT	PROVEN MUTANTS	
	MUTATIONS TO r^r	MUTATIONS TO R^g
47-102A.5-2	9/12,525	3/11,804
47-14.3-7	4/ 8,459	3/ 8,020
Total	13/20,984	6/19,824
Frequency per 10,000 gametes	6.2±1.7	3.0±1.2
POSSIBLE ADDITIONAL MUTANTS NOT FULLY TESTED		
1	1*	0
2	3†	2‡
Total, including uncertain cases	17/20,984	8/19,824
Frequency per 10,000 gametes	8.1±2.0	4.0±1.4

* Colorless seed, failed to germinate.

† One colorless seed, failed to germinate.
Two colorless seeds, colored seedling, but test against contamination not complete.

‡ Two colored seeds, colorless seedling, but test against contamination not complete.

tinguishable from R^g after the seedling stage. In the study of the action of these alleles, certain modifiers were found which intensify the action of the weak R^r alleles. One of these, tentatively designated Vr, in the presence of the weak R^r alleles, results in strong pigmentation in various tissues, including the mature leaf sheath, leaf blade, glumes, anthers, and pericarp. Another, Sr, results in strong pigmentation of the silk, although it has little effect upon the pigmentation of other tissues. Both Vr and Sr are entirely without effect upon pigmentation in plants of R^g or r^g genotype.

All the plant-color mutants identified in the present experiment were wholly devoid of plant color, in both seedling and mature stages. The six proven mutants were further tested for a possible low level of R^r action by

crossing each mutant to both *r^g Vr* and *r^g Sr*. Progenies of these crosses, closely examined in seedling and mature plant stages, showed no anthocyanin pigmentation in any plant tissue. All six mutants are phenotypically indistinguishable from *R^g*. They are designated *R^g*-1:Columbia, *R^g*-2:Columbia, etc., in further reference below, the first three mutants being those from gametes of 102A.5–2, and the other three those from 14.3–7. Cultures 102A and 14 were duplicate plantings from a single selfed ear of the Columbia stock.

Data on transmission through male and female germ cells are available for a number of the mutants identified in this experiment. They are fairly representative of transmission data in general for mutants of the various genes studied in the experiments on spontaneous mutation in maize. The mutants from this experiment were not systematically tested for transmission, but data on male transmission are available for most of them, and supplementary data on female transmission and on the segregation ratios in self-progenies for several of them. The data submitted are simply the progeny counts for *R* in the various types of crosses involving plants heterozygous for the mutant alleles, male transmission being indicated by the cross on *r^g*, female transmission by the cross by *r^g*, and the combined effect of male and female transmission by the result of self-fertilization.

Deviations from equality in the transmission of the contrasted alleles in such crosses are not necessarily due to differential viability of the *R* alleles concerned. Various factors reducing male transmission, by reducing the rate of pollen-tube growth or otherwise, are known, and there are undoubtedly other factors affecting transmission which may be present in some of the tester stocks. Any such factor linked with *R*, if present in the tested plant, would modify the *R* transmission ratio. No such factors are present in the *R^r*: Columbia stock or the *r^g* multiple recessive stock, as judged by *R* ratios in general in the crosses of these stocks with others. The original mutant plants are therefore free of such factors, but there is no assurance of their absence in the mutant heterozygotes produced by crossing the original mutant plants with other testers.

Transmission data for the mutants identified in this experiment are shown in table 2. Among the aleurone color mutants, designated *r^r*: Columbia, Nos. 1–9 inclusive are those from gametes of plants 102A.5–2 and Nos. 10–13 inclusive are those from gametes of plants 14.3–7. The *R^g*: Columbia mutants are similarly identified above.

The data give no indication of significant reduction of gametophytic transmission for any of the mutants tested. In some of the self-progenies there are deviations in the opposite direction. These are apparently due to factors for reduced transmission linked with the tester allele in some of the tester stocks, rather than to any advantage of the mutant *R* allele in competition. The direct transmission tests for mutants concerned (*r^r*-3, *r^r*-7, *R^g*-4, *R^g*-5) give no indication of increased transmission of the mutant allele. The data in general indicate that spontaneous mutation of *R^r* to *r^r* or to *R^g* is without appreciable effect upon viability or physiological efficiency.

TABLE 2

Transmission tests of Columbia mutants.

	MALE TRANSMISSION		FEMALE TRANSMISSION		SEGREGATION IN SELF-PROGENY	
Aleurone-color mutants:	($r^g \times R^g$ or r^g/r^r-mut.)		(R^g/r^r-mut. $\times r^g$)		(R/r^r-mut. ⊗)	
	R^g or r^g	r^r	R^g	r^r	R	r^r
r^r-1:Columbia					86	23
r^r-2:Columbia	129	117			52	25
r^r-3:Columbia	53	39			(201	30)*
r^r-5:Columbia	71	80			172	47
r^r-6:Columbia	117	95	169	178		
r^r-7:Columbia	15	7	165	117	(187	36)*
r^r-8:Columbia	144	153	84	83	329	105
r^r-9:Columbia	119	124	110	127		
r^r-11:Columbia	38	33				
r^r-12:Columbia	25	20				
r^r-13:Columbia	42	45				
Plant-color mutants:	($r^g \times R^g$-mut./r^{ch})				(R^r or r^{ch}/R^g-mut. ⊗)	
	R^g	r^{ch}			R^r or r^{ch}	R^g
R^g-1:Columbia			201	191	263	86
R^g-2:Columbia			180	173	248	170
R^g-3:Columbia	170	178	158	135	359	117
R^g-4:Columbia	166	173			224	123
R^g-5:Columbia	186	175	133	121	92	52
R^g-6:Columbia			71	83	54	20

* Progeny of R^g/r^r-mut. C/c ⊗, showing ratio of red-base to green-base seedlings. In these two cases the entry in the second column represents the frequency of R^g R^g individuals—that is, homozygotes of the non-mutant allele.

FREQUENCY OF SIMULTANEOUS LOSS OF ALEURONE-COLOR AND PLANT-COLOR EFFECT IN SPONTANEOUS MUTATION

Although independent loss of aleurone color and plant color is definitely the rule in spontaneous mutation or R^r, there are rare exceptions in which mutation occurs to the colorless seed, colorless plant allele, r^g. In the course of experiments with five R^r alleles of phenotype similar to R^r:Columbia, two such cases were found.

The R^r alleles used in these experiments include two (Cornell, Boone) derived from genetic tester stocks, two (Quapaw, Ponca) derived from stocks secured from Indian tribes, and one (Black) derived from the commercial popcorn variety Black Beauty. The data submitted include all data at hand for homozygous stocks of these alleles in which the incidence of aleurone-color mutation was determined in populations of female gametes, and in which the effect of the mutation upon plant color was determined. Among the 82 colorless aleurone mutations identified and confirmed in these experiments, all but one were mutations to r^r. The single exception was a mutant of R^r:Cornell,

identified as a single seed of colorless aleurone on a full-set ear of colored aleurone. This seed produced a plant entirely devoid of anthocyanin in all tissues. A sample of 20 of the colored seeds of the same ear was planted to confirm the constitution of the parent plant, and the 19 resulting plants all showed typical R^r coloration.

The mutant plant produced wholly normal pollen and a full-set ear, giving no indication of any gametophytic defect resu ting from deficiency. The plant could not be directly tested for male transmission, since it was r^g: Cornell/r^g in constitution. It was pollinated by R^r R^r, and 16 plants were grown from consecutive seeds in a region of perfect seed set on the ear. Half of these should be r^g: Cornell/R^r, the other half r^g/R^r, though the two classes are indistinguishable. Male transmission of R was determined for each of these 16 plants, and was found normal in all cases. It may be concluded therefore that if the mutation involved physical loss of the R^r gene, the deficiency had not sufficient effect upon gametophyte viability to cause a significant disadvantage in competitive pollen-tube growth.

There were, in addition in these cultures, almost a hundred presumable aleurone-color mutations which had not been given the routine confirmatory tests. Experience with some hundreds of colorless-aleurone mutations determined in female gametes has shown that failure of confirmation is extremely rare in R stocks with full aleurone color. These exceptions are probably ascribable to the rare occurrence of a mutation affecting the endosperm and not the embryo. Certain comparisons of mutation frequency in these stocks are therefore made with confirmatory tests on only a sample of the mutants identified.

The presumed mutant seeds were planted to identify any additional case of mutation to r^g. Among 79 seedlings resulting, one proved to be green and produced a wholly green plant. This mutant occurred in the allele R^r: Black, and, like the Cornell case, occurred as a single seed on a full-set ear of colored aleurone. Fifteen colored seeds planted yielded 13 plants, all of which were R^r in plant-color phenotype. The mutant plant in this case also produced pollen of normal appearance and a full-set ear. It was pollinated by R^r R^r for a test of male transmission, but this test has not yet been made.

The total frequency of mutation to r^g therefore was two among 161 mutations to colorless aleurone. The frequency observed in the various R^r alleles is shown in table 3.

The possibility that the r^g mutations may represent chance coincidences of independent mutation for aleurone color and for plant color is rather slight. The mutant r^g: Cornell occurred in a culture of the pure Cornell stock. The observed frequency of mutation to R^g in this stock is 2/2,997. R^r: Cornell in derivative strains extracted from crosses of the Cornell stock with other stocks has yielded mutations to R^g in a number of instances, the total frequency observed in these cases being 18/17,217. The mutant r^g: Black occurred in a derivative stock of homozygous R^r: Black extracted from a cross of the original Black stock with r^{ch}. The frequency of mutation to R^g observed in the same culture of this derivative stock was 1/3,342. None of the R alleles

TABLE 3

Plant-color phenotype of aleurone-color mutants of R^r.

R^r ALLELE	ALEURONE-COLOR MUTANTS		
	r^r	r^g	TOTAL
R^r:Cornell	88	1	89
R^r:Boone	41	0	41
R^r:Quapaw	19	0	19
R^r:Ponca	5	0	5
R^r:Black.	6	1	7
Total	159	2	161

tested yields mutations to R^g at a rate higher than that given above for R^r:Cornell, and the total frequency observed for R^r alleles of this group, tested in homozygous stocks, is 27/50,002. It would be expected therefore that by chance coincidence the frequency of mutations to r^g, among the total of aleurone-color mutations, would be less than one per thousand.

ABSENCE OF EFFECT OF ALEURONE-COLOR MUTATION UPON PLANT-COLOR PHENOTYPE

The foregoing results show that spontaneous mutations causing total loss of aleurone-color effect do not cause total loss of plant-color effect, and conversely that those causing total loss of plant color do not cause total loss of aleurone color. In the former case, in particular, it is possible to determine the degree of independence of the two components of action much more definitely, for the plant-color action of *R* alleles varies over a wide range and that of any two alleles may be compared with considerable precision. Does mutation involving aleurone color have any appreciable effect upon plant color?

It is possible to recognize very small differences in *R*-expression in plant color phenotype. In the study of allelic variability at the *R* locus previously mentioned (STADLER and FOGEL 1943, 1945, and unpublished) 22 R^r alleles, constituting a virtually unselected sample of R^r's of independent origin, were found to include few if any duplicates in phenotypic action. The alleles mentioned in the preceding section, Boone, Cornell, Quapaw, Ponca, and Black, are very similar in phenotypic effect, and affect the pigmentation of the same tissues in substantially similar ways. It is quite impossible to distinguish these alleles by examination of an individual plant. Yet there are differences in average level of pigmentation, and these may be objectively demonstrated by the classification of individual plants in plantings made from seed mixtures. These differences persist in parallel extractions of the R^r's after crossing and backcrossing repeatedly to a uniform r^g stock. Whether these differences are due to differences in the alleles or in factors modifying R^r-expression is for our present purpose beside the point; in either case the result shows that minute

differences in R^r-expression may be recognized and objectively demonstrated.

In the case of all r^r mutants grown for confirmatory tests, the mutant plant is compared in details of plant-color expression with a sample of ten unmutated sib plants, with detailed comparison in the seedling and the flowering stage. In almost all such comparisons the mutant falls within the range of variation of the unmutated sib plants, but since this range may sometimes be fairly wide, this comparison is not very significant. A much more sensitive test is the comparison of sib populations from the backcross R^r/r^r (mutant)$\times r^g$. Here populations of colored and colorless seeds, representing the parent and the mutant allele, may be directly compared in numbers sufficient to equalize variations due to genetic and environmental modifying factors. Such comparisons have been made with detailed examination in 23 cases involving mutants of various R^r alleles of this group, and including several cases selected on the basis of the first comparison as most likely to involve differences in plant-color phenotype. These show no significant difference in the plant-color phenotype of the mutant r^r and the parent R^r.

The effect of plant-color mutation upon the level of aleurone-color expression cannot be so closely checked, since differences in aleurone-color level are not subject to such close distinction. Ears of genotype R^r/R^g (mut.)$\times r^g$ have been examined for possible aleurone differences in the case of several R^g mutants. This examination was made in the large populations of such ears produced for the determination of aleurone-color mutability in the R^r and R^g-mut. alleles. In general aleurone-color development was too uniform to permit a separation for test. In five ears a possible segregation of a dilute aleurone was found, but the seedlings grown from full-colored and dilute-colored seeds showed no relation of aleurone-color dilution to the R locus. It is evident therefore that mutation of these R^r's has no appreciable effect upon the level of aleurone-color expression.

EFFECT OF PLANT-COLOR MUTATION UPON ALEURONE-COLOR MUTABILITY

Four R^g mutations from R^r-Cornell were crossed with the parent allele to produce cultures including plants of genotype R^r-Cornell/R^g-Cornell. In three cases, the cross was made between the original R^g mutant plant (R^g/r^g) and one or more unmutated sib plants (R^r/r^g); in the fourth case the mutant was crossed with plants of the pure R^r:Cornell stock. Mutation to colorless aleurone was then determined in all R^r/R^g plants of the four cultures, and all mutants were classified for plant color and carried through full confirmatory tests.

This trial provides a comparison of aleurone-color mutability in the R^r and its mutant R^g allele under practically identical conditions as to any environmental or genetic factors which may modify mutation frequency. The R^r and R^g alleles tested for mutation are present in equal numbers in each plant, and are present together in the same somatic cells until meiosis. The genes with which they are associated after meiosis are the same except for genes linked with R. Even as to genes of this linkage group they are virtually certain to be identical, since the chromosome bearing the mutant R^g is the

chromosome of the parent R^r stock modified only by the effects of the R mutation. Except for any unknown interaction between R^r and R^g, all mutations to r^r may be considered to represent exclusively mutations of the R^r allele, for in homozygous stocks the R^r allele mutates almost exclusively to r^r, and mutations of R^g to r^r are never found. Similarly, mutations to r^g may be considered to be almost exclusively from the R^g allele, though an occasional mutation of R^r to r^g may be expected.

The results are shown in table 4.

TABLE 4

Effect of plant color mutation upon seed color mutability.
F_1 Plants of R^r:Cornell/R^g:Cornell.

MUTANT	CULTURE	NUMBER OF SEEDLINGS EXAMINED	MUTANTS	
			r^r	r^g
R^g-10:Cornell	57-2011	2,573	2	0
R^g-11:Cornell	57-2012	1,625	1	0
R^g-13:Cornell	57-2013	5,272	4	1
R^g-14:Cornell	57-2141	4,423	5	0
Total		13,893	12	1

Of the 13 aleurone-color mutants found, all but one were r^r. Although the number of mutants is small, the deviation from equality is striking.

The single r^g mutant was a somewhat defective plant, and showed segregating defective pollen in the ratio of approximately 1:1. The presence of segregating defective pollen is quite unusual in untreated maize plants and has not previously been found in any of the spontaneous mutants. It may result from translocation or deficiency, and both of these have been found to occur spontaneously at low rates in maize. The fact that the r^g plant was distinctly defective in development suggests that the cause of the defective pollen was deficiency.

If the presumed r^g mutant was r^g in phenotype due to a segmental deficiency including the R locus, the parental chromosome affected may have been either the R^r or the R^g chromosome. In either case the plant is not to be included among the aleurone-color mutations. If the segregating defective pollen was due to deficiency in some other region, or to translocation, the plant represents a mutation to r^g. These possibilities may be further investigated by a progeny test.

If translocation not involving deficiency were responsible, the resulting semisterility would be transmitted to the progeny, normally to 50 percent of the progeny. If deficiency were responsible, the possibility of transmission would depend upon the degree of deficiency. Most deficiencies are non-transmissible, but deficiencies of minor degree may be transmitted through female germs cells in varying proportions. The degree of deficiency indicated by the

appearance of the plant and of the defective pollen was such as to suggest that the deficiency would not be transmitted.

The ear of the r^g plant was pollinated by R^r. It proved to be semisterile, indicating either translocation or non-transmissible deficiency. A progeny of 18 plants was grown, and it was found that all of these produced wholly normal pollen. This eliminates translocation as the cause of the semisterility and indicates that the plant was heterozygous for a lethal deficiency. It is not possible to determine whether this deficiency included the *R* locus. The plant therefore is indeterminate as to *R* mutation.

The ratio of mutants from R^r to mutants from R^g is thus either 12:1 or 12:0.

To insure that this inequality is due to a difference in mutability and not to a difference in the frequency of R^r and R^g gametes on the ears of R^r/R^g plants, a population of 80–100 seedlings was grown from each of the 11 ears on which the mutations occurred. All the ears proved to be R^r/R^g in constitution, and none deviated substantially from a 1:1 ratio.

It is thus evident that the frequency of aleurone-color mutation is substantially reduced following the occurrence of plant-color mutation in R^r: Cornell. This strongly indicates that the determiners of aleurone color and plant color are not independent genes. However, in view of the possible total absence of mutations of R^g in the data now at hand, an alternative mechanism could be considered, involving reduplication of the aleurone-color gene incidental to loss of the plant-color gene in the mutation of R^r to R^g. Further experiments are in progress to determine more precisely the degree of rarity of the aleurone-color mutations of R^g, the relative mutability of R^r and R^g in homozygotes and heterozygotes, and the converse effect of aleurone-color mutation upon plant-color mutability. The discussion of possible mechanisms underlying the effect will be deferred until the results of these experiments are available.

SUMMARY

The gene R^r (colored aleurone, colored plant) mutates spontaneously with appreciable frequency to two distinct forms, r^r (colorless aleurone, colored plant) and R^g (colored aleurone, colorless plant).

In two sib-plants of R^r: Columbia, tested extensively for frequency of *R* mutations among the male gametes, the frequencies of mutation to r^r and to R^g were of the same order (10^{-3}–10^{-4}).

No mutations to intermediate levels of plant-color effect were found, although the technique was such as to detect mutation to various known intermediate levels.

The spontaneous mutations to r^r and to R^g have no appreciable effect upon viability or physiological efficiency in haplophase, as judged by competitive pollen-tube growth in pollinations from heterozygous plants. The homozygous mutants are wholly normal in viability and development.

Spontaneous mutations to r^g, involving simultaneous loss of aleurone-color and plant-color effect, were very rare, but not so rare as would be expected if due only to chance coincidence of independent mutations for aleurone color and plant color.

The two spontaneous mutations to r^g observed were also without appreciable effect upon viability and physiological efficiency.

Aleurone-color mutation has no appreciable effect upon the level of plant-color expression, and plant-color mutation none upon the level of aleurone-color expression. In the former case very slight difference in level of gene action could be detected if present.

Plant-color mutation has a pronounced effect upon aleurone-color mutability of the gene or gene-complex concerned. This is shown by the much-lowered aleurone-color mutability of the mutant R^g allele, as compared with the parent R^r allele. This result is not consistent with the hypothesis that the r^r and R^g mutations represent independent alterations of distinct components of the gene or gene-complex R^r.

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DOMINIGENES OF THE VESTIGIAL SERIES IN DROSOPHILA MELANOGASTER

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IT IS almost axiomatic in studies of developmental genetics that the action of a particular gene cannot be described completely without reference to the residual genotype or "general genic environment" (term proposed by TSCHETWERIKOFF 1926). It is also generally agreed that "all factors probably partake of the nature of modifiers, since each factor appears to affect many characters and to alter the expression of other factors" (SINNOTT and DUNN 1932). The widespread acceptance of these concepts has led to the frequent assumption of the presence of modifiers on general grounds without a serious attempt to define the genetic basis more precisely. Modifiers whose presence has been only vaguely determined have even played an important role in certain theoretical generalizations, particularly in the field of evolutionary genetics. Few studies have been made with a view to a precise analysis of the genetics of a system of modifiers. The present investigation is an extension of one very thorough analysis of this type of situation—that is, the effect of a series of genetic modifiers on heterozygotes bearing the normally recessive gene vestigial in *Drosophila melanogaster*.

PREVIOUS WORK

In a series of papers, GOLDSCHMIDT and coworkers (GOLDSCHMIDT 1935, 1937; GOLDSCHMIDT and GARDNER 1942; GOLDSCHMIDT and HOENER 1937; GARDNER 1942) have presented a study of the effect of dominance modifiers ("dominigenes") on the action of genes for wing scalloping in *Drosophila melanogaster*, particularly on vestigial (*vg*) and its alleles. They used a modifier stock called "Doho," established by GOLDSCHMIDT in 1935 and maintained since that time by constant selection for notched-wing *vg* heterozygotes. Genetic analysis of Doho by GOLDSCHMIDT led to the following conclusions. Three dominigenes were present, one in the X chromosome, and one each in the second and third chromosomes. In the presence of both autosomal dominigenes in homozygous condition and the sex-linked dominigene in simplex condition in the males and duplex condition in the females, almost all flies heterozygous for *vg* showed scalloping or notching of the distal margin of the wings, a phenotype comparable to that characteristic of the lesser vestigial allele *vestigial-notched* (vg^{no}). Flies carrying only the recessive sex-linked dominigene manifested 1–2 percent notching. In the absence of the sex-linked gene, the "dominant" autosomal dominigenes together produced only 1–2 percent notched flies. The dominigenes had no visible effects other than their influence on the dominance of *vg*, except for a possible effect on viability. The sex-linked dominigene *do-X* was thought to be an allele of cut (*ct*). The second chromosome dominigene, named *B*, was thought to be closely linked to *vg*, and the

third chromosome dominigene, named *A*, was apparently closely linked to spineless (*ss*). Doho stock also contains the second chromosome gene purple (*pr*), introduced into the stock together with *B* and *vg* from the multiple second chromosome stock 2ple, and spineless (*ss*), introduced from a spineless stock together with *A*. The Doho stock was constituted to segregate for *vg* and was maintained by constant selection for the notched heterozygotes. Following a series of crosses whose varying results were explained by GOLDSCHMIDT (1937) as due to segregation for the second chromosome factor *B*, a dominigene stock with a high and relatively constant incidence of notching was obtained. This definitive Doho stock was declared to be homozygous for all three dominigenes.

When GARDNER (1942) obtained certain unexpected results from crosses involving Doho, continued homozygosity of the stock for all three dominigenes was brought under question. GARDNER crossed Doho vestigial females with normal males from an Oregon-R wild stock, expecting all the F_1 males to show the notched effect. (Curiously enough, there are no data available to support the assumption that all males of the genetic constitution *do-X*, *vg* $B/++$, $A/+$, as in the above cross, should be notched.) The actual results from single pair matings varied from 25 normal and 95 notched males in the culture showing greatest penetrance to 21 normal and four notched males in the culture showing least penetrance. The total for F_1 males of all crosses was 338 normal and 344 notched. The F_1 females, being heterozygous for *do-X*, fulfilled expectation in showing little penetrance of notching. GARDNER concluded that the dominigene stock was no longer completely homozygous for all three dominigenes. On using females from a selected dominigene stock in the cross Doho *vg* ♀ × Oregon-*R* ♂, he once again obtained a 1:1 ratio for normal to notched among the F_1 males, a result which he concluded was due to a condition of balanced heterozygosity for one of the dominigenes in the selected stock. This dominigene was tentatively assumed to be the second chromosome factor *B*. Thus the cross was reconstructed as *do-X*/*do-X*, *vgB*/*vg*+, *A*/*A* ♀ × + ♂, the notched F_1 males being of the constitution *do-X*, *vg* $B/++$, $A/+$, and the normal F_1 males being *do-X*, *vg*+/++, $A/+$. Since Doho stock was thus supposed to have changed genetically from its final status in GOLDSCHMIDT'S (1937) analysis, it seemed advisable to make a few appropriate crosses to test the stock for homozygosity of the dominigenes. A routine check by the present author led to a full-scale reanalysis of the dominigene stock.

PRELIMINARY CROSSES

The first step was a repetition of crosses of Doho flies *inter se*. In crosses of this sort, GOLDSCHMIDT obtained variable results which he attributed to segregation for dominigene *B*. After selection, he obtained high and relatively constant ratios of notched flies. In this stock assumed to be homozygous for the dominigenes, results from the cross notched × notched averaged 57.6 per cent notched for females, and 64.6 per cent notched for males. These values are in excess of the expected maximum of 50 percent *vg* heterozygotes, the conclusion being that the homozygotes showed lessened viability. Repetition

of this cross in the present study produced variable results which compared favorably with the higher range of values in GOLDSCHMIDT's earlier data but without values as high as those obtained from his "homozygous" stock. It may be seen in table 1 that notching varied from 32.4 percent to 53.8 percent for females and from 37.0 percent to 55.9 percent for males, with averages of

TABLE 1

Doho notched ♀ × *Doho notched* ♂.

CULTURE NO.	FEMALES			MALES		
	+	NOTCHED	vg	+	NOTCHED	vg
H8	13	23	8	11	23	17
H9	18	28	14	17	35	12
H10	15	28	9	19	19	16
H11	27	24	23	23	38	14
H12	26	28	16	18	26	22
H13	17	16	12	19	27	7
H14	19	23	21	18	35	12
H15	20	27	22	16	28	20
H51	29	29	20	16	37	14
H58	14	24	7	16	26	11
H8-1	20	16	14	16	23	14
H8-2	14	23	13	14	22	20
H8-3	23	42	13	25	37	13
H8-4	23	30	15	14	35	16
H8-5	23	22	15	12	23	10
H8-7	22	33	13	14	33	12
H8-8	24	42	21	15	29	19
H8-9	14	17	12	17	18	9
H8-10	16	13	10	14	19	9
H8-11	9	11	11	6	11	7
H8-12	15	22	7	15	21	15
H10-1	22	39	12	17	43	17
H14-1	19	19	6	16	21	11
H58-1	14	13	13	10	14	5
Totals	456	592	357	378	643	322

43.1 percent for females and 47.9 percent for males. GOLDSCHMIDT's results varied from 8.9 percent to 75.0 percent for females and from 17.8 percent to 78.6 percent for males. Both experiments were conducted under presumably the same environmental circumstances, the offspring of single pair matings being raised at 25°C in half-pint bottles containing 50–60 cc of the usual corn-meal-molasses-agar yeast medium, with counts terminated on the 18th day after mating.

Two conclusions may be drawn. (1) A change has taken place in the Doho stock, leading either to a lessened dominigene effect or to a negation of the lowered viability among homozygotes observed in GOLDSCHMIDT's data. (2)

If segregation is occurring in the Doho stock used in the present experiments, it is for a dominigene or dominigene complex with smaller effects than those involved in GOLDSCHMIDT's crosses.

Attempts to subdivide the cultures of table 1 into a number of subgroups on any scheme involving segregation of a single factor, such as the classification employed by GOLDSCHMIDT in his 1937 paper, proved arbitrary and uncertain. Appropriate statistical treatment fails to demonstrate any lack of homogeneity among the cultures. For the data as a whole, the sum of chi-squares equals 99.843 with 115 degrees of freedom, with no individual chi-square value exceeding the 5 percent level of probability. For the sexes considered separately, the sum of chi-squares with 46 degrees of freedom equals 45.471 for the females and 40.730 for the males. Again, no chi-square value exceeds the 5 percent level.

The results of the chi-square test indicate merely that the data are not inconsistent with a concept of homogeneity and that the variation in the data may be attributed to chance. Other possibilities, however, cannot be excluded. Not only may various factors in the external environment play a modulating role, but also random segregation for one or more dominigenes with minor effects may still be involved.

That the stock culture of Doho was not completely homozygous became apparent in outcrosses of *vg* Doho females with wild type males. The picture is somewhat obscured by the presence of further genetic factors in the wild stocks themselves.

In an attempt to reproduce as exactly as possible GARDNER's crosses, Oregon-R was the first wild stock used. Results from single pair matings of *vg* Doho ♀ × Oregon-R ♂ varied from 0.0 to 50.0 percent notched females and from 20.0 to 75.4 percent notched males. Not only was the penetrance of notching quite variable among the males, but the *do-X*/+ females, which had conformed to expectation in GARDNER's crosses by showing few notched wings, now tended to show a definite, though variable, penetrance of the notched character. Furthermore, backcrosses of F_1 males from a culture showing extreme results (♀ ♀ 33+ : 24 notched; ♂ ♂ 15+ : 46 notched), to notched females from Doho stock indicated that the Oregon-R stock contained at least one modifying factor in the third chromosome.

The data indicating the presence of this dominance modifier in Oregon-R stock are shown in table 2. These data are presented in the form of two sets or classes of contrasting results, class I including the total offspring of 11 single pair matings, and class II of ten single pair matings. The division into two classes was based on the following considerations.

In a number of cultures, non-spineless and spineless flies showed a comparable penetrance of notching. Since the cross was putatively *do-X/do-X, vg pr B/+ pr B, ss A/ss A* ♀ × *dc-X, vg pr B/+ + +, ss A/+ +* ♂, the genotype of the spineless flies was supposely *AA*, and of the non-spineless flies, *Aa*. As concluded by GOLDSCHMIDT, *AA* flies should exceed *Aa* flies in penetrance of notching. The presence of an additional notching intensifier was apparently involved in some of the F_1 crosses under consideration.

That segregation for one or more modifiers of notching penetrance was involved in these crosses was further indicated by a statistical analysis of the data. When all non-spineless, non-vestigial flies in each culture were classified simply as normal or notched and the chi-square test applied, the sum of chi-squares was found to be 123.241 with 20 degrees of freedom. Individual chi-

TABLE 2

Doho notched pr ss ♀ × F_1 *notched* ♂ [*Doho vg pr ss* ♀ × *Oregon-R* ♂ (*H88*)] (*offspring with vg wings not included*).

CLASS	+		*ss*		*pr*		*pr ss*	
	TOTAL FLIES	PERCENTAGE NOTCHED	TOTAL FLIES	PERCENTAGE NOTCHED	TOTAL FLIES	PERCENTAGE NOTCHED	TOTAL FLIES	PERCENTAGE NOTCHED
				Females				
I	271	46.1	227	46.3	133	90.2	106	97.2
II	243	11.5	247	38.9	114	62.3	98	91.8
				Males				
I	309	47.6	266	46.2	111	93.7	112	98.2
II	235	22.6	229	49.8	97	77.3	97	97.9

square values for nine of the 21 cultures exceeded the 5 percent level of probability, six of these values being beyond the 1 percent level. The cultures probably do not represent a homogeneous population.

Division of the cultures into two groups, with the mean penetrance of notching for all cultures as an arbitrary point of division, gave class I with a notching penetrance of 60.2 percent for non-spineless, non-vestigial flies and a sum of chi-squares of 10.355 with ten degrees of freedom, and class II with a notching penetrance of 33.9 percent and a sum of chi-squares of 9.589 with nine degrees of freedom. No culture showed a chi-square value in excess of the 5 percent level. For each of these classes, a concept of homogeneity is acceptable.

The spineless and non-spineless flies of class II show the expected relationship as regards penetrance of notching for *AA* and *Aa* flies, but the two groups show comparable, and, therefore, unexpected, results in class I. If the two third chromosomes in the original Oregon-R male parent differed in their effect on the penetrance of notching, the two classes of results among the F_1 crosses finds a ready explanation. The original cross may be tentatively reconstructed as *do-X/do-X, vg pr B/vg pr B, ss A/ss A* ♀ × +, +/+, *Intensifier*/+ ♂. Indirect evidence that this was the situation has been received from MR. M. KODANI who shortly afterward found in another connection that the Oregon-R stock used was no longer normal but contained a few chromosomal abnormalities, including an inversion in the right arm of chromosome III similar to *In(3R)Payne*. It has been claimed by GARDNER (1942) (also *vide infra*) that *In(3R)P* acts as an intensifier of the dominigene effect. It is pos-

sible that this was the major intensifying factor present in the above crosses.

That one or more additional factors may have contributed in a lesser degree to heterogeneity among the cultures was indicated by the results for the spineless flies. When all spineless, non-vestigial flies in each culture were classified simply as normal and notched and the chi-square test applied, the sum of chi-squares was found to be 33.391 with 20 degrees of freedom, P=.031. Values for four of the cultures exceeded the 5 percent level, none of the values being in excess of the 1 percent level. For class I, the sum of chi-squares was 21.319 with ten degrees of freedom, P=.019, with two cultures showing chi-square values in excess of the 5 percent level. The possibility of heterogeneity has even been slightly increased for the cultures of class I. For class II, the sum of chi-squares was 10.780 with nine degrees of freedom, P=.290, with one culture showing a chi-square value in excess of the 5 percent level. Since the spineless flies do not contain an Oregon-R third chromosome, any heterogeneity indicated by these data must be attributed either to the effect of undetermined environmental fluctuation or to segregation for one or more modifying genes present in at least one of the other chromosomes involved in the cross (assuming, of course, that the results are not due to chance). Segregation in Doho stock was indicated as a causative factor by the following series of crosses.

Since the Oregon-R stock had been shown to be segregating for one or more dominance modifiers, the cross Doho *vg* ♀ × wild type ♂ was repeated with males obtained from Canton and Florida wild type stocks. The specific lines used were Canton-S, certified by C. B. BRIDGES as showing a normal salivary pattern, and Florida $+_{10}$ isogenic 1939 from AMHERST COLLEGE, which had been inbred for several generations in 1939, but which remained in a balanced heterozygous condition (or had once again become heterozygous) for *In(3R)P*. These males were crossed with females from the original Doho stock and from a Doho line made homozygous by appropriate crossing with balancer stocks (*vide infra* on method of establishing isochromosomal stocks). The results from the four types of matings are presented in table 3.

The first cross, Doho *vg* ♀ from the original Doho stock × Canton-S ♂, showed an average notching penetrance among the F_1 males of 34.4 percent. A chi-square test did not indicate a lack of homogeneity among the cultures involved, although one culture had $\chi^2=3.390$, P=.066. (Two cultures with exceptional results are not included here but will be discussed shortly.) Repetition of the cross with females from homozygous Doho stock (table 3, cross 2) provided an average notching penetrance among F_1 males of 44.0 percent. These cultures also satisfied a test for homogeneity. Comparison of the two sets of data, however, gave a chi-square value of 4.022, indicating a probability of less than .05 that the two groups of cultures were genetically identical. Since presumably no change had taken place in the Canton-S stock, the data favor a conclusion of heterozygosity for dominance modifiers in the original Doho stock.

Results of the crosses with Florida males are even more suggestive of segregation in the original Doho stock. When the female parents were obtained from

this line (table 3, cross 3), statistical analysis of the data for the F_1 males showed a probability of homogeneity among the cultures of less than .001. Chi-square values for three of the eight cultures were beyond the 5 percent level, one being beyond the 1 percent level. Average notching penetrance was 61.8 percent for males and 6.6 percent for females. When the female parents were obtained from the homozygous line (table 3, cross 4), the probability of homogeneity among the cultures rose to .53. Average notching penetrance also rose, the values obtained being 75.8 percent for males and 24.2 percent for females. A reanalysis of the cultures of cross 3 on the assumption that 75.8 percent notched males was the expected value showed four of the eight cultures in close agreement, one culture with a chi-square value in excess of the 5 percent level, and three cultures with chi-square values far in excess of the 1 percent level.

TABLE 3

Doho vg ♀ ×*wild type* ♂.

CROSS	FEMALES		MALES		χ^2 (MALES)	df	P (MALES)
	TOTAL FLIES	PERCENTAGE NOTCHED	TOTAL FLIES	PERCENTAGE NOTCHED			
1. Doho *vg* (original line) ♀ ×Canton-S ♂	250	1.6	256	34.4	4.603	6	.59
2. Doho *vg* (homozygous line) ♀ ×Canton-S ♂	308	1.9	250	44.0	1.908	4	.74
3. Doho *vg* (original line) ♀ ×Florida ♂	427	6.6	434	61.8	29.381	7	<.001
4. Doho *vg* (homozygous line) ♀ ×Florida ♂	426	14.1	401	75.8	6.066	7	.53

GARDNER'S (1942) results from the same cross are of interest. Average notching penetrance for males in his cultures was 60.4 percent. Chi-square tests showed two cultures which deviated significantly from a 1:1 expectancy. Average notching penetrance for the F_1 males in these cultures was 70.5 percent. GARDNER attributed his results to segregation for *In(3R)P* in the Florida stock. From the present data, it would seem that GARDNER'S Doho stock was not yet homozygous for all dominance modifiers when he made the cross with Florida stock. (The present author is of the impression that almost all flies of the Florida stock were of the constitution *In(3R)P*/lethal. This point, however, was not adequately tested.)

Further evidence for segregation in the original dominigene stock was afforded by the effect of selection on the results of the cross Doho *vg*×Doho+. Three sets of this cross were made: with parents from unselected Doho stock, with F_1 flies from the single pair mating H8 (*cf.* table 1), and with Doho *vg* females from an inbred homozygous stock (*vide infra*) and Doho+ males from a line derived from cross H8. As may be seen in table 4, the effect of inbreeding

was to raise the percentage of notched flies, maximum values being obtained by the use of a stock known to be homozygous.

It has already been mentioned that in the cross Doho *vg* (original line) ♀ × Canton-S ♂, two cultures with unexpected results were obtained. These showed an average notching penetrance for F_1 males of 86.8 percent, which is more than twice the value for the remaining cultures. (The data for the

TABLE 4

Doho vg × Doho+ from different lines.

CROSS	FEMALES		MALES	
	TOTAL FLIES	PERCENTAGE NOTCHED	TOTAL FLIES	PERCENTAGE NOTCHED
1. Doho *vg* × Doho+ from unselected stock	472	75.0	462	92.2
2. Doho *vg* × Doho+ F_1 flies of Cross H8	116	85.3	117	95.7
3. Doho *vg* ♀ (homozygous) × Doho+ ♂ (H8 line)	153	96.1	131	99.2

males were 12+:79 notched, and for the females, 95+:4 notched.) Backcrosses of F_1 males from these aberrant cultures to Doho *vg* females are summarized in table 5, class I. The fact that notching penetrance among the nonspineless exceeded the penetrance among the spineless flies (64.5 percent as against 49.4 percent for females and 85.5 percent as against 69.2 percent for males) indicated that the Canton-S parents contributed a third ($+^{ss}$) chromosome containing a dominigene whose effect with one dose of *A* was greater

TABLE 5

Doho vg ♀ × F_1 ♂ (*Doho vg* ♀ × *Canton-S* ♂).
(*Offspring with vg wings not listed.*)

CLASS	FEMALES				MALES			
	+		*ss*		+		*ss*	
	TOTAL FLIES	PERCENTAGE NOTCHED	TOTAL FLIES	PERCENTAGE NOTCHED	TOTAL FLIES	PERCENTAGE NOTCHED	TOTAL FLIES	PERCENTAGE NOTCHED
I	76	64.5	77	49.4	83	85.5	65	69.2
II	85	29.4	77	51.9	91	45.1	78	62.8

than the effect of *AA*. Data from comparable backcrosses in which the F_1 males were obtained from cultures with "expected" results are summarized in class II of table 5. The result expected in the absence of third chromosome Canton-S dominigenes with major effects is obtained: the spineless (*AA*) flies exceed the nonspineless (*Aa*) in notching penetrance.

DEVELOPMENT OF ISOCHROMOSOMAL STOCKS

The demonstration of genetic heterogeneity in the wild type stocks used made it apparent that for further analysis all stocks used should be made isochromosomal—that is, isogenic except for the chromosome(s) to be tested. This was done with the aid of balanced lethals according to a scheme similar to that demonstrated by PLOUGH (1942) for the detection of autosomal mutations. The derivation of the isochromosomal stocks is illustrated by the examples shown in figure 1. It should be noted that a source of heterogeneity is introduced by the chance of crossing over in the fourth chromosome, which contains no crossover inhibitor, and by the rare crossovers involving the chromosomes carrying the inversions.

All the stocks thus established derived their dominigene chromosomes from the same Doho chromosomes, their wild type chromosomes from identical Canton-S chromosomes, and their chromosomes bearing vestigial or its alleles from single chromosomes from Doho stock and a series of stocks containing genes of the vestigial series.

The term isochromosomal has been used advisedly. To say that two stocks are isogenic except for a particular locus or loci implies that all other loci are known to be identical for the two stocks compared. However, the Doho and Canton-S chromosomes may not only differ by the alleles known or suspected to be present, but they may involve other, as yet unidentified, genetic factors. Furthermore the various vestigial and vestigial-allele chromosomes may also involve one or more added dominigenes. Increased isogeneity would require extensive selective backcrosses among the isochromosomal stocks.

ACTION OF THE DOMINIGENE CHROMOSOMES

With all chromosomes now identified as to origin, it was possible to assess accurately the penetrance value of the dominigene chromosomes in different combinations. When the appropriate crosses were made, considerable variation from culture to culture was obtained for genetically identical matings. Inspection of the data showed that mass matings, with a consequent increase in number of offspring and probable larval crowding and greater competition for food, tended to produce a greater percentage of notched flies. This effect had previously been noted by GOLDSCHMIDT (1935). The values presented in figures 2 and 3, in which the data have been summarized without regard to this variability, may differ somewhat from results obtained from single pair matings only.

The histograms of figure 2 show the effect of dominigene chromosomes in various combinations on the penetrance of heterozygous vestigial from Doho stock. All females are homozygous, and all males, hemizygous, for the Doho X chromosome. Genetic constitution as regards the second and third chromosomes is indicated by GOLDSCHMIDT's symbols for the autosomal dominigenes —that is, *B* for the second chromosome from Doho, *A* for Doho III, and *b* and *a* for the corresponding Canton-S chromosomes bearing the normal alleles. Since the *vg* chromosome is derived from Doho stock in this series of crosses, all flies are either *Bb* or *BB*, depending on whether the other second chromo-

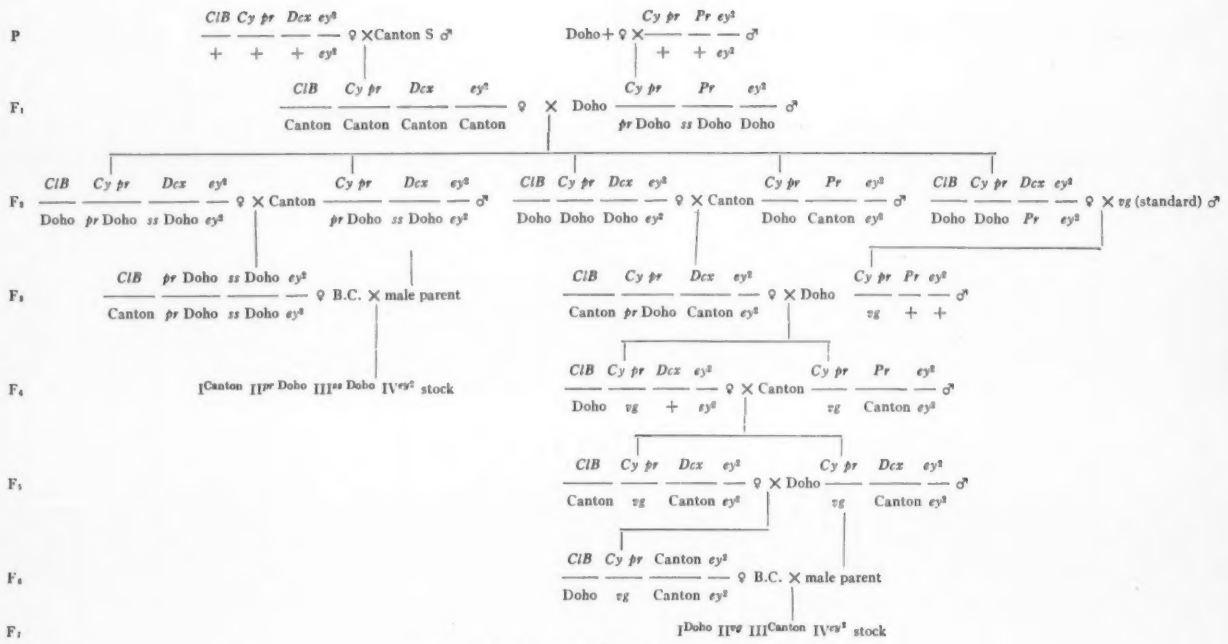
P
F1
F2
F3
F4
F5
F6
F7
ClB Cy pr Dcx ey2 / + + + ey2 ♀ × Canton S ♂
Doho + ♀ × Cy pr Pr ey2 / + + ey2 ♂
ClB Cy pr Dcx ey2 / Canton Canton Canton Canton ♀ × Doho Cy pr Pr ey2 / pr Doho ss Doho Doho ♂
ClB Cy pr Dcx ey2 / Doho pr Doho ss Doho ey2 ♀ × Canton Cy pr Dcx ey2 / pr Doho ss Doho ey2 ♂
ClB Cy pr Dcx ey2 / Doho Doho Doho ey2 ♀ × Canton Cy pr Pr ey2 / Doho Canton ey2 ♂
ClB Cy pr Dcx ey2 / Doho Doho Pr ey2 ♀ × vg (standard) ♂
ClB pr Doho ss Doho ey2 / Canton pr Doho ss Doho ey2 ♀ B.C. × male parent
I Canton II pr Doho III ss Doho IV ey2 stock
ClB Cy pr Dcx ey2 / Canton pr Doho Canton ey2 ♀ × Doho Cy pr Pr ey2 / vg + + ♂
ClB Cy pr Dcx ey2 / Doho vg + ey2 ♀ × Canton Cy pr Pr ey2 / vg Canton ey2 ♂
ClB Cy pr Dcx ey2 / Canton vg Canton ey2 ♀ × Doho Cy pr Dcx ey2 / vg Canton ey2 ♂
ClB Cy pr Canton ey2 / Doho vg Canton ey2 ♀ B.C. × male parent
I Doho II vg III Canton IV ey2 stock

FIGURE 1.—Method of establishing isochromosomal stocks.

some is derived from Canton-S or from Doho. As regards the third chromosome, there are three combinations, *aa*, *Aa*, and *AA*. Totals for each combination are included in the legend to figure 2. Separate histograms have been prepared for females and males.

Notching penetrance in the data of figure 2 varies from 3.8 percent for females and 17.0 percent for males when one Doho second chromosome is present to 89.9 percent for females and 96.2 percent for males in the presence of four autosomal chromosomes bearing dominigenes. An increase in pene-

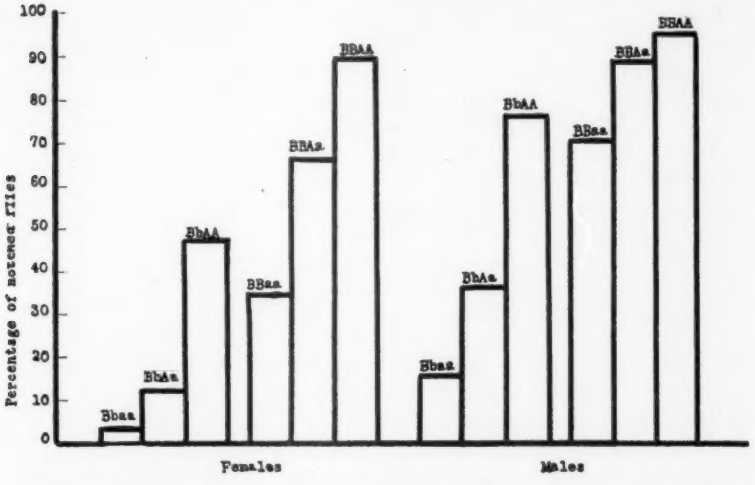

FIGURE 2.—The effect of the autosomal dominigenes *B* and *A* in different combinations on heterozygous vestigial from Doho stock. The X chromosomes are from Doho. The total number of flies involved are distributed as follows:

♀♀ *Bbaa* 421, *BbAa* 365, *BbAA* 370, *BBaa* 256, *BBAa* 280, *BBAA* 336.
♂♂ *Bbaa* 429, *BbAa* 291, *BbAA* 408, *BBaa* 256, *BBAa* 269, *BBAA* 317.

trance is associated with each additional Doho chromosome. An indication of the nature of this effect is given by the observation that a more than additive action is involved. The effect of one second chromosome (*Bbaa*) is 3.8 percent for females and 17.0 percent for males. The effect of adding a Doho third chromosome (*BbAa*) is the further manifestation of notching in 9.1 percent of the remaining females and 24.2 percent of the remaining males. On the assumption that *A* and *B* act independently of each other, that there is no interaction, the values 9.1 percent and 24.2 percent are constant increments associated with the presence of *A* in a single dose. A comparison of *BBaa* and *BBAa* flies as regards the penetrance of notching gives new values to *A* of 48.8 percent for females and 62.6 percent for males. The expected value for *BBAa* females is 41.1 percent notched [35.2 percent+9.1 percent ×(100 percent−35.2 percent)], and the actual value is 66.8 percent. The

effect of *A* on the penetrance of notching is dependent on the number of Doho second chromosomes present. *A* and *B* interact.

On the simplest assumption that only two autosomal dominigenes, *A* and *B*, are involved, the two loci act as pseudo-alleles (*cf.* CREW and LAMY 1932). On the assumption that not two but many modifiers are involved, each Doho chromosome contributes a number of polygenes (*cf.* MATHER 1941 *et seq.*) each of which has a small but definite effect on the dominance of vestigial. Whichever assumption is correct, the observed factor interaction implies that the same or closely related developmental processes are being affected.

Further conclusions may be drawn as to the effects of dominichromosomes II and III. According to GOLDSCHMIDT (1937), both *A* and *B* must be present in at least one dose each, in addition to dominigene *do-X*, in order to produce notched wings. The present data show that in the absence of Doho III, a considerable penetrance effect may be observed. Females of the constitution *do-X/do-X*, *vg B/+*, *+/+* show an incidence of notching of 3.7 percent, while 17.5 percent of the *do-X*, *vg B/+*, *+/+* males are notched.

The effect of Doho II is greater than that of Doho III. *do-X/do-X*, *vg B/+B*, *A/+* females show an incidence of notching of 69.1 percent, while the value for *do-X/do-X*, *vg B/++*, *A/A* females is only 49.0 percent. The corresponding values for the males are 88.9 percent and 79.8 percent.

Males of the genetic constitution *do-X*, *vg B/++*, *A/+* (*BbAa*) show a notching penetrance of 37.1 percent, which does not differ significantly from the value of 44.0 percent obtained for males from the cross Doho *vg* (homozygous line) ♀ × Canton-S ♂ ($\chi^2 = 2.673$, $P = .10$). The average value of 40.3 percent is less than the 50 percent value obtained by GARDNER from the cross *vg* Doho ♀ × Oregon-R ♂. Since both experiments were conducted under approximately the same environmental conditions, two alternative explanations may be suggested: either GARDNER's Oregon-R stock differed genetically (as regards dominigenes) from the Canton-S stock used in the present experiment, or the selected Doho stocks were not identical. The similarity in the results obtained by GARDNER and by the present author from the cross Doho *vg* × Florida (*vide supra*) indicates that the Doho stocks were comparable in the two experiments, and the difference under discussion may be attributed to a genetic difference between Canton-S and Oregon-R.

It is apparent from the data of the present experiment, and from the fact that there is no evidence to the contrary, that GARDNER's results of 50 percent instead of 100 percent notched F_1 males is not inconsistent with a concept of homozygosity for the dominigene stock. Therefore, his assumption of segregation of Doho stock for dominigene *B* is unnecessary.

THE "STANDARD" VG STOCK

Certain unexpected results were obtained by the use of a chromosome bearing vestigial from "standard" *vg* stock—that is, a *vg* stock not known to carry dominigenes—instead of a *vg* Doho chromosome (fig. 3). In the presence of one Doho second chromosome, the penetrance of notching was 11.5 percent for females and 34.2 percent for males as compared with the previously ob-

tained values of 3.7 percent and 17.5 percent, respectively. In the presence of one second and one third Doho chromosome, the values are 40.5 percent and 62.1 percent as compared with 15.5 percent and 44.8 percent. For one second and two third chromosomes, similar differences were obtained—namely, 57.9 percent and 82.5 percent as compared with 49.0 percent and 79.8 percent.

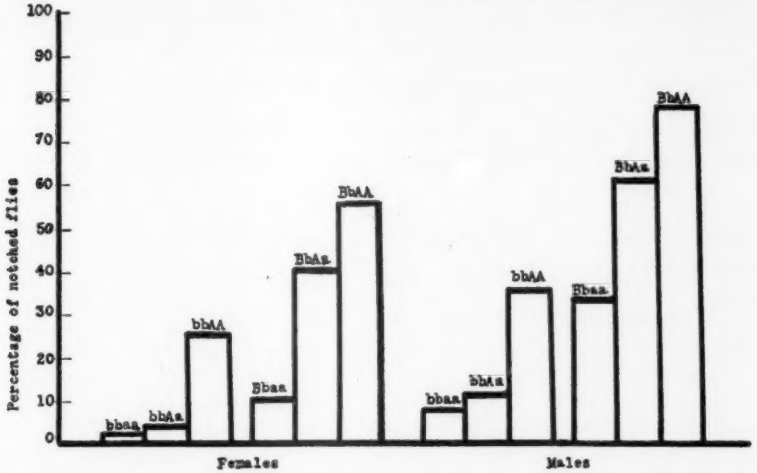

FIGURE 3.—The effect of the autosomal dominigenes *B* and *A* in different combinations on heterozygous vestigial from standard University of California stock. The X chromosomes are from Doho. The total number of flies involved are distributed as follows:

♀ ♀ *bbaa* 168, *bbAa* 141, *bbAA* 109, *Bbaa* 236, *BbAa* 973, *BbAA* 184.
♂ ♂ *bbaa* 156, *bbAa* 124, *bbAA* 124, *Bbaa* 273, *BbAa* 913, *BbAA* 163.

In the presence of Doho chromosomes in various combinations, *vg* from THE UNIVERSITY OF CALIFORNIA stock shows a potency different from that of *vg* from Doho stock. Yet the two vestigials are otherwise indistinguishable from each other. This type of situation is characteristic of the iso-alleles (STERN and SCHAEFFER 1943) or cryptic alleles (GOLDSCHMIDT 1944), which may be distinguished from each other only by specific genetic (or environmental) tests. The two chromosomes in question may differ from each other as regards the vestigial locus itself or with regard to modifying factors more or less closely linked to *vg* (in addition to *B*). If there are dominigenes present which are so closely linked to vestigial that *vg* and its modifiers act substantially as a unit, it would be difficult to demonstrate that the differences present do not involve the vestigial locus itself. That this may be the explanation of iso-alleles (and perhaps other members of some of the allelic series?) has been recognized as a possibility by STERN and SCHAEFFER.

Except for the difference in strength between the *vg* Doho and *vg* standard chromosomes, the data presented in figure 3 parallel the results previously reported. The difference in strength between Doho II and Doho III is again

shown, with the values for *bbAa* flies being 4.4 percent for females and 12.1 percent for males as contrasted with 11.2 percent and 34.1 percent, respectively, for *Bbaa* flies. As to dominigene interaction, the values for *BbAa* flies exceed considerably the sum of the individual values for *Bbaa* and *bbAa* flies (that is, 40.5 percent versus 15.1 percent for females and 62.1 percent versus 42.1 percent for males). The result is similar when *BbAA* flies are compared with *Bbaa* and *bbAA* flies (that is, 56.5 percent versus 34.0 percent for females and 78.5 percent versus 58.0 percent for males).

DOMINIGENE ACTION OF DIFFERENT VG STOCKS

A subsequent test of the dominigene action of a number of vestigial stocks produced the results shown in table 6. The lines used were: (1) vestigial from

TABLE 6

Doho+ ♀ × vg ♂. Single pair matings.

VESTIGIAL STOCK	FEMALES		MALES	
	TOTAL FLIES	PERCENTAGE NOTCHED	TOTAL FLIES	PERCENTAGE NOTCHED
vg-Amherst	455	14.9	416	39.7
sca vg	60	1.7	60	43.3
vg-Univ. of California (1)	492	9.1	557	32.0
(2)	408	14.2	409	54.3
(3)	220	16.4	228	52.6
b vg (inbred)	324	4.3	330	30.0
b vg (outcrossed)	335	14.9	324	77.5
vg bw (1)	313	8.6	326	35.3
(2)	235	16.6	211	69.7

AMHERST COLLEGE (originally obtained from M. H. HARNLY), which had been inbred by single pair matings for several generations in 1940 and 1941, and which was not known to carry dominigenes; (2) *sca vg* from CALIFORNIA INSTITUTE OF TECHNOLOGY, inbred in 1941; (3) *vg* from THE UNIVERSITY OF CALIFORNIA AT BERKELEY; (4) an inbred black vestigial stock from YALE UNIVERSITY; (5) a black vestigial stock from YALE UNIVERSITY, derived from inbred stock outcrossed to Oregon-R stock and subsequently recovered, and (6) a vestigial brown stock from AMHERST COLLEGE, which had been derived from an Amherst *vg* stock, and which had shown variable penetrance of notching in outcrosses (*cf.* vg^{40b} *bw*, Amherst #38, D.I.S. 14).

F_1 males of the cross Doho+ ♀ × *vg*-Amherst ♂ show a notching penetrance of 39.7 percent, which is comparable to the values previously obtained for a

putatively similar complex of dominigenes—that is, 37.1 percent in figure 2 and 44.0 percent in table 3. The presence of further autosomal dominigenes is not indicated. The heterozygous do-X females, however, show a penetrance value of 14.4 percent which is equivalent to the value of 12.6 percent for *do-X/do-X, vg B/+, A ss/+* females in figure 2 and far in excess of the previously obtained value of 1.9 percent (*cf.* table 3). One or more dominigenes are probably present in the X chromosome of *vg*-Amherst stock.

Data from a backcross of F_1 ♂♂ of this cross to Doho+ ♀♀ are presented in table 7. The values resulting from this mass mating will be seen to be somewhat higher than the comparable data of table 6. This increase in notching penetrance, applying to all data in table 7 which can be compared with previously obtained values, is an illustration of the effect of larval crowding mentioned earlier.

TABLE 7

Doho+ ♀♀ × F_1 ♂♂ (*Doho+* ♀♀ × *vg* ♂♂). *Mass matings.*

VESTIGIAL STOCK	FEMALES				MALES			
	+		*ss*		+		*ss*	
	TOTAL FLIES	PERCENTAGE NOTCHED	TOTAL FLIES	PERCENTAGE NOTCHED	TOTAL FLIES	PERCENTAGE NOTCHED	TOTAL FLIES	PERCENTAGE NOTCHED
vg-Amherst	304	24.0	274	66.4	290	51.4	247	76.9
vg-Univ. of California	202	26.2	175	61.1	211	57.3	164	76.2
b vg (inbred)	238	20.6	187	71.1	189	33.9	176	80.1
b vg (outcrossed)	300	73.0	208	68.3	248	81.5	223	70.9
vg bw	234	47.9	221	54.3	264	61.0	211	60.7

The cross Doho+ ♀ × *sca vg* ♂ produced penetrance values which conformed to expectation—that is, 1.7 percent for females and 43.3 per cent for males. A backcross test was not made.

Three sets of data are presented for the cross Doho+ ♀ × *vg*-University of California ♂. The first two series of crosses were made early in the analysis; the third, at the same time as the remaining crosses of table 6. The female parents of the first set were derived from the unselected dominigene stock; those of the second set were F_1 flies from the cross H8; and those of the third were from the Doho+line of the isochromosomal series of stocks. The results of the cross Doho+ (unselected) ♀ × *vg*-U.C. ♂ show relatively weak penetrance and thus fall in line with the previously reported data from crosses involving the unselected dominigene stock (*cf.* tables 3 and 4). The other two sets of crosses give comparable results, the males showing an average penetrance of 53.4 percent. (This is lower than the previously obtained figure of 62.1 percent in figure 2, but the latter value includes the results of two mass matings with unusually high penetrance. With the exclusion of these exceptional data, the value in figure 2 would be 52.7 percent.) However, the females of set 1 with 9.1 percent and of the other two sets with the higher average

value of 13.6 percent show a greater incidence than that expected for *do-X*/+ flies, though lower than the 30.4 percent value previously obtained for *do-X*/*do-X*, *vg*-U.C./Doho II, Doho III/+females. The reciprocal cross, *vg*-U.C. ♀ ×Doho+ ♂, also gave unusually high results—namely, 15.6 percent (293+:54 notched) for females and 19.2 percent (257+:61 notched) for males, when the male parents were derived from the isochromosomal stock, and the lower values 11.1 percent (184+:23 notched) for females and 13.1 percent (161+:36 notched) for males, when the male parents were derived from H8 stock. Of three possible explanations for these results, two may be discounted. It is unlikely that the enhancement in values is due to presence of further dominigenes in the third or fourth chromosomes of the vestigial stock, since in the absence of these suspect chromosomes, *do-X*, *vg*-U.C./Doho II, Doho III/+ males show as high a penetrance of notching as do the males in this cross. If the known enhancing action of the second chromosome of *vg*-U.C. stock were entirely responsible, the value of 19.2 percent for putatively +, *vg*-U.C./Doho II, Doho III/+ males would suggest a higher differential between the values for *do-X*, *vg*-Amherst/Doho II, Doho III/+ and *do-X*, *vg*.-U.C./Doho II, Doho III/+ males than that actually obtained. The backcross data shown in table 7 provide evidence of a similar nature against these two hypotheses. It seems most probable, therefore, that the X chromosome of *vg*-U.C. stock has an enhancing action.

The cross Doho+ ♀ ×*b vg* inbred ♂ (table 6) shows little penetrance for the F_1 females and the relatively low value of 30.0 percent notched for the F_1 males. The inhibition of dominance in the presence of the gene for black body has been frequently noted (*cf.* GARDNER 1942, for the effect of *b* on heterozygous *vg*, and DEXTER 1914, for its effect on Beaded (*Bd*)). The present results, then, are not unexpected. Similar data were obtained from the backcross (table 7) for Doho III/+ flies.

Unexpected results were forthcoming, however, when this cross was repeated with males from the outcrossed *b vg* stock (table 6). Notching penetrance rose to 77.5 percent for the males and 14.9 percent for the females, indicating the presence of an intensifier of dominance in one of the autosomes of the *b vg* stock. The presence of such a factor in the third chromosome is shown in the backcross data of table 7 where Doho III/? flies exceed Doho III/Doho III flies in notching penetrance, indicating an intensifying action of the "normal" third chromosome.

One further cross of this type was made, namely Doho+ ♀ ×*vg bw* (Amherst) ♂ (table 6). The cultures fell into two distinct groups, one giving 8.6 percent notched females and 35.3 percent notched males, and the other, 16.6 percent and 69.7 percent respectively. The backcross data (table 7) show that Doho III/+? and Doho III/Doho III flies do not differ significantly in notching penetrance. Segregation for a third chromosome intensifier in *vg bw* stock is indicated.

GARDNER obtained from the cross Doho *vg* ♀ ×*bw* ♂ the relatively high penetrance values of 13.0 percent for females (107+:16 notched) and 80.5 percent for males (23+:95 notched). His conclusion that *bw* had an enhanc-

ing effect on notching penetrance has not been borne out by the present results. The values for F_1 females are higher than expected for Doho I/+, Doho II/*vg*, Doho III/+ flies, indicating an intensification of the dominigene effect. As for the males, the 69.7 percent value is explained by the presence of the third chromosome intensifier, while the 35.3 percent value obtained in the probable absence of this intensifier is even below that expected for Doho I, Doho II/*vg*, Doho III/+ flies. The data indicate the presence of a sex-linked dominigene as well as the third chromosome modifier but do not show any intensifying action for the *vg bw* chromosome.

Further evidence for segregation in *vg bw* stock is forthcoming in another connection. In an investigation of the effects on wing scalloping of different compounds of the vestigial series, it was found that *vg bw*/vg^{no} wings varied in pattern from normal to vestigial at 25°C, while *vg*-Amherst/vg^{no} wings showed an intermediate and relatively constant series of forms. Moreover, single pair matings of the cross *vg bw* ♀ × vg^{no} ♂ gave widely different results, a further indication of segregation. Both intensifying and inhibiting factors would seem to be involved.

(A comparison of the action of these factors with that of GOLDSCHMIDT's dominigenes is of interest. In testing the effects of the dominigenes of Doho stock on the pattern of scalloping in compounds of the vestigial alleles, GOLDSCHMIDT and HOENER (1937) obtained inconclusive results except for the penetrance of distal notching in the least scalloped types. Further tests by the present author failed to produce definite results. However, inversions in the third chromosome show a striking effect on the expressivity of wing scalloping (*cf.* GOLDSCHMIDT and GARDNER 1942, for discussion of the evidence). These inversions also act as enhancers of penetrance (GARDNER 1942, and the further evidence on the effect of *In(3R)P* discussed above). It may be suggested that modifiers of the inversion type effectively alter developmental processes at relatively early stages, while the modifiers of penetrance (and expressivity) of distal notching are restricted in effect to the period just before final determination of wing form.)

A final demonstration that the vestigial stocks themselves contained modifiers is provided by the results of the cross *vg* ♀ ♀ × Canton-S ♂ ♂ (table 8). Only one of the stocks—that is, *vg bw*—contained a mutant previously suspected to have an intensifying action. Yet two other stocks, those from AMHERST and THE UNIVERSITY OF CALIFORNIA, also showed a definite penetrance of notching in the heterozygous F_1 males.

vg ALLELES AND DEFICIENCIES

An effect of the dominigenes on other members of the vestigial series has also been observed. GOLDSCHMIDT and HOENER (1937) found that the dominigenes increased the penetrance of notching in those vestigial alleles, which ordinarily in heterozygous or homozygous condition or in various combinations showed little or no notching. The alleles tested were vg^{ni} (vestigial-nicked), vg^{no} (vestigial-notched), and vg^{nw} (vestigial-no wing). To expand this series, the present author tested heterozygotes of the extreme alleles vg^{nw}

TABLE 8

vg ♀ × *Canton-S* ♂.

VESTIGIAL STOCK	FEMALES		MALES	
	TOTAL FLIES	PERCENTAGE NOTCHED	TOTAL FLIES	PERCENTAGE NOTCHED
vg-Amherst	128	0.0	127	11.8
vg-Univ. of California	136	0.0	136	22.1
sca vg	106	0.0	99	0.0
b vg (inbred)	153	0.0	138	0.7
b vg (outcrossed)	66	0.0	68	1.5
vg bw	157	3.2	292	5.8

and $vg^{No.2}$ (vestigial-Notch 2) and the deficiencies vg^{D} (vestigial-Depilate), vg^{C} (vestigial-Carved), vg^{I} (vestigial-Incised), and vg^{S} (vestigial-Snipped). Isochromosomal stocks were used throughout. The results in table 9 show that in every case the expected increase in notching in the presence of dominigenes was obtained.

TABLE 9

The effect of the first and third chromosomes from Doho on heterozygotes of vestigial alleles and deficiencies raised at 25°C.

ALLELE OR DEFICIENCY	CHROMOSOMES FROM DOHO	FEMALES		MALES	
		TOTAL FLIES	PERCENTAGE NOTCHED	TOTAL FLIES	PERCENTAGE NOTCHED
vg^{S}	I III	41	97.6	52	100.0
	none	103	19.4	132	41.7
vg^{I}	I III	45	84.4	39	100.0
	none	81	2.5	89	22.5
vg^{C}	I	8	12.5	12	33.3
	III	10	10.0	11	9.1
	none	192	2.1	214	4.7
vg^{D}	I	107	23.4	79	63.3
	III	144	6.3	102	20.6
	none	165	0.0	184	2.7
$vg^{No.2}$	I III	66	77.3	51	98.0
	III	27	3.7	25	8.0
	none	85	4.7	101	5.9
vg^{nw}	I III	81	85.2	92	100.0
	I	73	43.8	97	86.6
	III	102	5.9	90	41.1
	none	75	2.7	80	8.8

Crosses involving vg^{nw} show a greater notching penetrance than the comparable crosses with the supposedly more extreme allele $vg^{No.\ 2}$. On making compounds of these alleles and deficiencies with vg^{no}, it was found that $vg^{No.\ 2}/vg^{no}$ wing patterns were more extreme than those of vg^{nw}/vg^{no} flies, but less extreme than those of *vg*-Deficiency/vg^{no} flies. It may be concluded that $vg^{No.\ 2}$ is a more extreme allele than vg^{nw}, but that the vg^{nw} second chromosome contains a further dominance modifier, which, like the dominigenes from Doho stock, increases the penetrance (and expressivity) of notching of the distal margin of the wing but which has no observable effect on wing scalloping in the more extreme types.

The four deficiencies tested showed distinctly different notching potencies. For comparable crosses, all flies were isochromosomal except for the second chromosomes with the deficiencies (and, in part, for the fourth chromosome). It is clear, then, that the differences in penetrance are most likely attributes of the different second chromosomes. Since none of the deficiencies are identical, the simplest hypothesis would be the suggestion that the extent of each deficiency in itself affects the degree of penetrance. In other words, the loci adjacent to *vg* may themselves be modifiers—that is, inhibitors—of the dominance of *vg*, with a consequent shift in dominance in their absence. Such a hypothesis would have a possible bearing on concepts of the evolution of dominance, non-random assortment of genes, and the nature of allelic series.

THE IDENTITY OF THE DOMINIGENES

It was pointed out in the introductory remarks that Doho stock is homozygous for purple (*pr*) in the second chromosome and for spineless (*ss*) in the third chromosome. The possibile identity of these genes with GOLDSCHMIDT'S dominigenes *A* and *B* is indicated by several observations. (1) GOLDSCHMIDT (1935) derived the autosomal modifying factors from stocks containing *pr* and *ss* and introduced the latter genes together with *A* and *B* into his Doho stock. He also found that *A* and *ss* are very closely linked and that *B* and *vg* are probably closely linked. (GOLDSCHMIDT has mentioned in his 1935 paper that he is partially color-blind. His inability to distinguish between certain shades of red probably explains his failure to discuss linkage relationships between *B* and *pr*.) (2) GARDNER (1942) found that *pr* itself had a dominigene action. (3) Although GARDNER (1942) failed to observe any dominigene action for ss^a (spineless!), GOLDSCHMIDT (1935, 1937) and GOLDSCHMIDT and GARDNER (1942) found a definite enhancement of the notching effect in the presence of *ss*.

The present author decided to make a further test of the dominigene action of these mutants. A new *pr ss* stock was constructed from appropriate crosses of a *pr* stock and a *ss* stock obtained from YALE UNIVERSITY, which were supposedly only distantly related to the stocks previously used. Since the Doho *vg* line no longer existed, a new line was constructed by the introduction into Doho+ stock of *vg* from AMHERST. The crosses Doho *vg pr ss* ♀ × F_1 (Canton-S ♀ × *pr ss* ♂) ♂ and Doho *vg pr ss* ♀ × F_1 (Canton-S ♀ × Doho + ♂) ♂ were then made and the results compared. As may be seen from the

data of table 10, row 3, both chromosomes 2 and 3 of the new *pr ss* stock had a dominigene action, the values for notched males being 28.6 percent in the presence of II^{pr}, 36.0 percent in the presence of III^{ss}, and 64.1 percent in the presence of both chromosomes tested. In the absence of II^{pr} and III^{ss}, the value obtained was 20.0 percent. Although the total numbers of flies are too small to give significant differences throughout, the trend is unmistakable; both II^{pr} and III^{ss} have an enhancing action on the penetrance of notching. In the light of the previous evidence, it may be concluded that *pr* and *ss* are themselves dominigenes.

As expected from previous data, addition of $II^{pr\ Doho}$ and $III^{ss\ Doho}$ chromosomes also increase penetrance (table 10, row 4), the values being 46.4 percent,

TABLE 10

Doho vg (Amherst) ♀ × ♂ *pr*/+ *ss*/+.

MALE PARENTS FROM CROSS	+		*pr*		*ss*		*pr ss*	
	TOTAL FLIES	PERCENTAGE NOTCHED	TOTAL FLIES	PERCENTAGE NOTCHED	TOTAL FLIES	PERCENTAGE NOTCHED	TOTAL FLIES	PERCENTAGE NOTCHED
				Females				
Canton-S ♀ × Yale *pr ss* ♂	53	0.0	46	0.0	56	0.0	44	4.5
Canton-S ♀ × Doho *pr ss* ♂	115	0.0	100	0.0	110	0.9	112	2.7
				Males				
Canton-S ♀ × Yale *pr ss* ♂	40	20.0	42	28.6	50	36.0	39	64.1
Canton-S ♀ × Doho *pr ss* ♂	127	33.1	112	46.4	129	64.3	113	90.3

64.3 percent, and 90.3 percent, as against 33.1 percent for I^{Doho}, $II^{vg\ pr\ Doho}/+$, $III^{ss\ Doho}/+$ males. However, the values obtained are somewhat lower than the comparable figures of figure 2—namely, 89.2 percent, 76.5 percent, 96.2 percent, and 37.1 percent. This lowering of penetrance finds an explanation in a putative difference between the *vg* Doho chromosomes used in the two sets of crosses. As stated above, the new *vg* Doho chromosome was constructed by crossing *vg*-Amherst into the Doho stock. The *vg pr* crossover flies selected to establish the new stock may differ from the original Doho *vg pr* flies as regards loci close to *vg* for which there was no selection and which may not have been involved in the crossover. That this explanation is correct is shown by the change in strength of the $II^{Doho\ vg}$ chromosome. Formerly (*cf.* figures 2 and 3), II^{Doho} exceeded III^{Doho} in effect on penetrance; now the opposite is true.

It is clear from the above that the original *vg* Doho chromosome contained at least two dominigenes, one of which was probably *pr* itself, while the other was an "unseen" modifier more closely linked to *vg*. Thus GOLDSCHMIDT'S

description of *B* is correct for part of a dominigene complex in the second chromosome. On the basis of the present data, the possibility that *A* is also a dominigene complex, involving one or more dominance modifiers in addition to *ss*, cannot be excluded.

One further point must be discussed before proceeding. The penetrance values for males in table 10, row 3, are consistently lower than the comparable data in row 4. That this is also true for the genetically identical I^{Doho}, $II^{vg\ pr\ Doho}/+$, $III^{ss\ Doho}/+$ flies suggests that the shift in values cannot be entirely ascribed to a supposed difference in strength between the autosomes of different origin. Examination of the raw data shows that the figures of each cross in table 16 represent the summarized results of nine single pair matings. The average number of flies per culture for cross 1 is 41, and for cross 2, 102. The effect of environmental differences due to population density is strongly indicated.

GOLDSCHMIDT's identification of the X chromosome dominigene as an allele of cut (*ct*) has already been mentioned. His view rests on two lines of evidence. From the results of a crossover test, GOLDSCHMIDT (1935) had tentatively located this dominigene between *y* and *v*, roughly in the region of *ct*. To test the possibility that *do-X* and *ct* were actually alleles, he subsequently (1937) made a series of crosses between Doho females containing *vg* and ct^6 males. Practically all F_1 females heterozygous for *vg* were notched. Since *do-X*/+, *vg B*/+, *A*/+ females are notched in only about 1 percent of the individuals, he concluded that the F_1 females from these crosses were of the constitution ct^{do-x}/ct^6, *vgB*/++, *A*/+.

GOLDSCHMIDT's conclusions in both experiments are open to question. In the crossover test, he used the marker genes yellow, vermilion, and forked (*y v f*). His crossover data show definitely that *f*, or some closely linked modifier, has a strong enhancing action on the penetrance of notching. A reanalysis of his data indicates only that, assuming that *do-X* is a single point mutant and not a complex of modifiers, the X chromosome dominigene is at least nine crossover units to the right of *y* (locus = 0.) and at least four units to the left of *v* (locus = 33.0). The location of *do-X* in the neighborhood of cut (locus = 20.0) is still to be proven.

Two objections may be leveled at GOLDSCHMIDT's demonstration that *do-X* and ct^6 are alleles. In the first place, the effect of interaction of the dominigenes shown in the present paper might in itself be sufficient to explain an increased penetrance in the presence of two non-allelic sex-linked dominigenes, *do-X* and ct^6. Secondly, an examination of GOLDSCHMIDT's results shows that practically all F_1 males of the putative genetic constitution *do-X*, *vg B*/++, *A*/+ in his crosses with ct^6 are also notched. Since it is now known that only 50 percent of the males of this constitution are expected to show notching, the conclusion is inescapable that the ct^6 stock contains one or more autosomal notching enhancers.

In this connection, GARDNER's (1942) results with facet (*fa*) are of interest. From the cross Doho *vg* ♀ × *fa* ♂, he obtained results similar to those of GOLDSCHMIDT on using ct^6. Loath to dispute the supposed allelism between ct^6 and

do-X, GARDNER concluded that a factor interaction between the non-allelic genes *do-X* and *fa* was involved. However, the F_1 males of his crosses also showed an approximately 100 percent notching effect, indicating the presence of autosomal modifiers in the *fa* stock. Thus, both the cut and facet stocks contain enhancers of notching which are independent of the mutant loci known to be present (accumulated perhaps as a result of the usual method of maintenance of laboratory stocks by selection for strong phenotypes).

DISCUSSION

From the results of crosses with Oregon-R, Florida, and Canton-S wild stocks and various *vg* stocks, as well as the crosses of GOLDSCHMIDT and GARDNER referred to above, it is clear that dominance modifiers of vestigial are ubiquitous. Some of these modifiers have apparently no effect other than that on the penetrance of notching, a situation which led GOLDSCHMIDT to coin the term dominigene. Others, such as *pr* and *ss*, are genes with specific visible effects in addition to their influence on the dominance of vestigial. However, the possibility that "unseen" dominigenes may be present in a mutant stock raises the question whether a possible dominigene action of that stock is due solely to the specific "visible" mutant involved. Thus many of GARDNER'S (1942) conclusions regarding the dominigene action of a number of point mutants may have to be qualified after further tests. Particularly his contradictory results for the alleles *h* and h^2 and *bs* and bs^2 may stem from genetic dissimilarity for one or more unseen modifiers. On the basis of corroborative evidence, the following mutants may be assumed to act as dominigenes: the intensifiers *pr* (GOLDSCHMIDT 1935, 1937; GARDNER 1942; and the present paper) and *ss* (GOLDSCHMIDT 1935, 1937; GOLDSCHMIDT and GARDNER 1942; and the present paper), and the inhibitor *b* (DEXTER 1914; GARDNER 1942; and the present paper).

In studies on the effect of selection on "normal" or wild-type bristle number in *Drosophila melanogaster*, MATHER and coworkers (MATHER 1941, 1942, 1943, 1944; MATHER and WIGAN 1942; SISMANIDIS 1942; WIGAN 1941, 1944) have observed the presence in all the major chromosomes of numerous "invisible" mutants with minor modifying effects. These modifiers have been called polygenes in contradistinction to "visible" mutants, which have been variously termed oligogenes (MATHER 1942), genes with major effect (MATHER 1944), and genes with specific effect (SLIZYNSKI 1945). As a result of his investigation on the modifying effect of various Y chromosomes, MATHER (1944) has gone even farther in this distinction by suggesting that the polygenes are heterochromatic and thus of a simpler chemical structure, while the typical laboratory gene or oligogene is euchromatic. Of the several possible objections to this line of reasoning, only those which stem from the *vg*-dominigene analysis will be discussed here.

Study of the effect of dominigenes on the expression of notching in the wings is basically the same problem as that of the effect of polygenes on bristle number. It is true that MATHER, SISMANIDIS, and WIGAN have been studying the effect of modifiers in the absence of oligogenes known to affect

bristle number, but GOLDSCHMIDT (1935, 1937) has performed a precisely comparable experiment in his studies of the effects of dominigenes in the absence of vestigial and its alleles. In fact, the presence of notching in the wings of "normal" flies indicates that dominance of wild over vestigial in the classical sense is not involved, but that the penetrance of notching may itself be considered an attribute of normality, similar to the distribution of "normal" phenotypes with more bristles or fewer bristles in the presence of the polygenes. An even closer parallel is obtained if expressivity of the notching effect is studied by giving values to different degrees of notching, as has been done by GOLDSCHMIDT (1935 *et seq.*) and GARDNER (1942).

The essential difference between the work of MATHER and his colleagues and the present investigation resides in the relative lability of the phenotypes studied. Thus MATHER has studied variation in number of ventral abdominal chaetae, a character which is relatively variable in natural material. On the other hand, SISMANIDIS has drawn similar conclusions from a study of variation of an almost invariable character, the number of scutellar bristles. In the present investigation, a relatively invariable character in natural material, normality of wing margin, has been made more labile by the presence of the gene *vg* in heterozygous condition.

Under these circumstances, the dominigene complex appears as a typical polygene situation. The parallelisms are several. Both "types" of modifiers are numerous. They are present in all the major chromosomes. They act in both directions—that is, dominigenes with enhancing and inhibiting actions and polygenes with plus and minus effects. Both groups of modifiers show the phenomenon of interaction.

There is one apparent difference. Although many dominigenes are "invisible" except for their effect on notching, genes with major effects—for example, *pr*, *ss*, and *b*—also act as dominance modifiers. If MATHER and his coworkers had investigated the possibilities of a similar situation for bristle number, they might well have found evidence of the same nature. On the basis of the dominigene work, there would seem to be no such basic difference between polygenes and oligogenes as that suggested by MATHER.

Genes which affect two or more apparently unrelated processes—for example, "visible" mutants which at the same time affect the degree of notching of the wing—are ordinarily referred to as pleiotropic. The usual inference is that the pleiotropic gene is directly involved in two or more developmental processes which may be relatively independent or causally related (*cf.* GRÜNEBERG 1938, for "real" vs. "spurious" pleiotropism). A particular circumstance, however, surrounds the manifold action of the "visible" dominigenes. On the one hand, the genes *pr*, *ss*, and *b* affect distinct characters—namely, eye color, size of bristles, body color; on the other, they apparently interact to affect the same or closely related processes concerned with notching of the wing. It seems unlikely that all these genes are directly involved in the major processes leading to determination of wing form (in so far as any distinction can be made among genes as to their relative importance in a particular series of reactions). However, they may all produce the same end result by affecting

the time relationships among other developmental processes—for example, by altering total time of development. Thus, all mutants could conceivably affect a number of end products, simply by changing the circumstances under which the processes leading directly to these end products operate. (The work of DOBZHANSKY and HOLZ 1943, in which mutants at three out of six loci were shown to affect spermatheca shape, may be another example of this sort.) On this basis, many examples of pleiotropism may fall into a special case of the action of gene modifiers.

The hypothesis of alteration in time relationships of developmental processes may be overly simple. On the other hand, the likelihood of some such explanation being a correct one is strengthened by a number of pertinent lines of evidence. (1) The duration of the egg-larval stage of homozygous vestigial flies is longer than that of heterozygous vestigial flies (HARNLY 1929) and of homozygous wild type flies (ALPATOV 1930). (2) The duration of the egg-larval stage of *ss*, *f*, and *vg pr* flies is longer than that of wild type flies (GOLDSCHMIDT 1935). (3) Prolongation of larval development by starvation of the larvae leads to an increase in notching (BRAUN 1939, 1942). (4) In the presence of either of the Minutes, *M(2)l²* and *M(3) w*, or of the duplication *T1305*, all of which prolong developmental time, the incidence of notching in the wings of *vg* heterozygotes is significantly increased (GREEN and OLIVER 1940). (5) Males show a greater degree of notching than do females (the present investigation, and the work of GOLDSCHMIDT and his collaborators), while the females exceed the males for more extremely scalloped types (HARNLY and HARNLY 1935, 1936; BLANC 1945). In the light of GOLDSCHMIDT's hypothesis that notching of the wing is produced by a lytic process initiated after pupation and that more extreme scalloping involves an earlier inception of lysis, this reversal in effect of sex on wing pattern affords a suggestive parallel to POWSNER's (1935) evidence that females have a longer egg-larval age and a shorter pupal age at 25°C as compared with males. Proof of the validity of the suggested hypothesis as to the mode of action of the dominigenes awaits further investigation.

SUMMARY

Reanalysis of GOLDSCHMIDT's vestigial-dominigene (Doho) stock has indicated segregation in Doho for one or more otherwise unidentified dominance modifiers of vestigial.

GARDNER's results from outcrosses of Doho with normal stocks have been shown to be compatible with homozygosity of Doho for all three of GOLDSCHMIDT's dominigenes, thereby obviating GARDNER's assumption of balanced heterozygosity for one of these dominigenes.

The demonstration of the ubiquity of dominigenes in wild type stocks has led to the construction of isochromosomal stocks for use in further crosses.

The observed interaction of GOLDSCHMIDT's second (*B*) and third chromosome (*A*) dominigenes indicates that the same or closely related developmental processes are affected.

It has been further observed that *B* is effective in altering the dominance of

vestigial in the absence of *A*, and *vice versa*, and that *B* exceeds *A* in effect on penetrance of wing notching in *vg*-heterozygotes.

The heightened notching penetrance in the presence of vestigial from the standard University of California stock is considered in the light of possible introduction of further dominigenes from the standard stock and the question of iso-allelism in the vestigial series.

Further crosses with a number of vestigial stocks demonstrated the presence of additional dominigenes in most of these stocks.

GOLDSCHMIDT and HOENER's investigation of the effect of the dominigenes on alleles of the vestigial series has been amplified by the use of further alleles, as well as deficiencies, of vestigial.

Further consideration of the dominigenes has led to the partial identification of GOLDSCHMIDT's second chromosome "gene" *B* with *pr* (purple) and third chromosome "gene" *A* with *ss* (spineless).

GOLDSCHMIDT's identification of the X-chromosome dominigene *do-X* as an allele of *ct* (cut) has been shown to be inconclusive.

Demonstration of the ubiquity of "unseen" dominance modifiers has led to a criticism of GARDNER's conclusions regarding the dominigene action of a number of point mutants.

MATHER's distinction between polygenes and oligogenes has been disputed on the basis of the parallelisms between dominigenes and polygenes and the apparent identity of *B* and *A* with the visible mutants *pr* and *ss*.

A hypothesis involving alteration in time relationships of developmental processes is presented as a tentative explanation of the mode of action of the dominigenes.

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INHERITANCE IN NICOTIANA TABACUM. XXI. THE MECHANISM OF CHROMOSOME SUBSTITUTION

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A CYTOGENETIC basis for virus resistance in Holmes Samsoun tobacco was analyzed recently in two papers by the author (GERSTEL 1943, 1945a). This variety had been produced by HOLMES (1938) by transferring resistance to the mosaic disease from *Nicotiana glutinosa* to *N. Tabacum*. This was accomplished by successive backcrosses for several generations of the amphidiploid to N. *Tabacum* (table 4). It was found that HOLMES had accomplished the substitution of a chromosome from *N. glutinosa*, which carried the factor or factors for mosaic resistance, for one of *N. Tabacum*. A study of the circumstances which make such a substitution possible will be the subject of this paper.

Substitution is possible in the progeny of plants which carry one or several *N. glutinosa* chromosomes in addition to a full complement of 24 *N. Tabacum* bivalents. Such a condition prevails in the first backcross from the amphidiploid *N. Tabacum-glutinosa* to *N. Tabacum*, and also in further backcross generations to *N. Tabacum*. On *a priori* grounds it may be suggested that a substitution can be attained by the following means: (1) Formation of trivalents between two *Tabacum* and one *glutinosa* chromosome. From such an association the *N. glutinosa* chromosome may enter alone one of the cells resulting from the first meiotic division, while the other would receive both or one of the *N. Tabacum* chromosomes. (2) Failure of the two *N. Tabacum* homologues to pair in metaphase may also lead to the formation of gametes containing only 23 tobacco chromosomes and one (or more) from *N. glutinosa*. This may be caused by an occasional conjugation between a *Tabacum* and a *glutinosa* chromosome in prophase leaving a *Tabacum* chromosome out of the association. Such an association may or may not fall apart in metaphase. (3) Division of unpaired chromosomes during the first reduction division has been frequently observed in Nicotiana. Sometimes this is followed by the inclusion of both split products in the daughter cell. In our case this process may lead to the formation first of gametes, and then of zygotes, containing a pair of the same *N. glutinosa* chromosome in addition to a full *N. Tabacum* complement. It was found, however, that such plants with 25II breed true and do not lose through non-conjunction a pair of *N. Tabacum* chromosomes (GERSTEL 1945b). This third process would result, therefore, in an "addition race" and not in a "substitution race." Hence, it will not be considered further.

First metaphases of plants having *N. glutinosa* chromosomes in addition to 24 *N. Tabacum* bivalents were studied to obtain evidence as to whether either or both of the first two processes described above occur. First metaphases of meiosis of pentaploid plants, containing two genomes of various

strains of *N. Tabacum* and one genome of *N. glutinosa*, were studied at first. Though it was believed that not all the cells seen contained 24 bivalents and 12 univalents, it was by no means possible to be certain. In a few cases cells with fewer than 24 pairs and a correspondingly increased number of univalents were observed, and in others trivalents. These counts, however, were believed to be unreliable in this irregular material, and it was not possible to get a large number of good preparations.

A simpler situation prevails in plants having but one *N. glutinosa* chromosome in addition to two *N. Tabacum* complements. Such plants were obtained by backcrossing the pentaploid and the following generation to normal tobacco. "Purpurea" was the tobacco variety used throughout as the *N. Tabacum* parent. Tests for mosaic resistance of the maternal parent at each step insured that the single *N. glutinosa* chromosome selected was the one which carried the disease resistance.

Twenty-five metaphase plates of each of four such plants were studied (table 1). Ninety-three of these 100 plates showed 24 pairs and one univalent.

TABLE 1

Metaphase figures of necrotic trisomics.

PLANT NO.	24II+1I	23II+3I	23I+1III	TOTAL
43 6p3	24	—	1	25
43 6p4	24	1	—	25
44 7p6	22	1	2	25
44 8p10	23	0	2	25
Total	93	2	5	100

Two plates, however, contained 23II+3I, and five plates showed what appeared to be a trivalent in addition to 23 pairs. These deviating cells (7 percent of the total) represented the material which may have led to the formation of substitution gametes. Some caution, however, is indicated in accepting this frequency. In both cells with 23II+3I two univalents were lying close together, possibly as a result of early disjunction, and it was not at all certain that such a behavior would result in abnormal gametes. Regarding the five cells with trivalents, it must be stated that metaphase chromosomes in tobacco are very much condensed. What appeared to be trivalents were large units, but these may have represented a univalent in proximity to a bivalent, perhaps in a different plane, rather than a conjugated trivalent. No chain trivalents were seen.

In order to obtain genetical evidence as to the mechanism of chromosome substitution, two types of tests were executed. First the breeding behavior of the trisomics was studied (table 2). For this purpose normal Purpurea was fertilized with pollen from two different trisomic plants. Two hundred seeds were planted from each cross; from these a total of 349 young plants resulted, of which 49 were mosaic resistant. About one-half of these were lost by disease

before they reached maturity, but the pollen fertility of the remaining 25 plants was studied. All but one showed the high degree of fertility which was also characteristic of the trisomic parent and were therefore trisomics themselves—that is, they had 24 *N. Tabacum* pairs and one *N. glutinosa* univalent. The one remaining plant had a great deal of aborted pollen—namely, about 35 percent. It also had one non-conjunctive pair of chromosomes—that is, 23II+2I. Pollen from this plant was applied to normal Purpurea. It segregated for normal type and resistant plants; but the proportion did not fit the expected 1:1 ratio. (The actual values were 18 resistant:49 normal, $P<.01$.) Metaphases of some of the resistant segregants showed again 23II+2I. The parent plant presumably was a substitution heterozygote, obtained in 1/25 of the necrotic survivors from the cross normal×trisomic. Its male parental gamete, therefore, had 24 chromosomes, one of which was of *N. glutinosa* origin.

TABLE 2

Backcrosses with trisomic necrotic plants: Purpurea×24 II+1 I.

TRISOMIC POLLEN PARENT	SEEDS PLANTED	PLANTS TESTED	MOSAIC RESISTANT	MOSAIC RESISTANT SURVIVED	VALENCIES	
					24II+1I	23II+2I
44 6p3	200	170	17	8	8	0
44 6p4	200	179	32	17	16	1
Total	400	349	49	25	24	1

Next a pentaploid plant with 60 chromosomes of the constitution *N. Tabacum-Tabacum-glutinosa* was crossed to *N. Tabacum* (table 3). In this experiment the white race of Purpurea, differing in a single Mendelian factor from normal Purpurea, was used as the recurrent *N. Tabacum* parent. The progeny segregated for mosaic resistance versus susceptibility, for pink versus white and for a number of less well defined characters due to the inclusion of *N. glutinosa* chromosomes carrying these factors in a part of the progeny. From 49 seeds sown, 31 plants reached the flowering stage. Good metaphase preparations were obtained from 30 of those in large enough number to be certain of the correctness of the chromosome counts. At least five good plates, but usually more, were counted from each plant. Three plants had only 23 pairs, not 24, and a varying number of univalents (plants No. 1, 27, 28). This is evidence for the irregularity of meiotic behavior in the pentaploid. Either the presence of *N. glutinosa* univalents in this plant interfered with conjugation of *N. Tabacum* pairs or multivalent associations resulted in irregular distribution of the *N. Tabacum* chromosomes in anaphase. Thus ovules were formed which contained only 23 *N. Tabacum* chromosomes and, in our three cases, additional *N. glutinosa* chromosomes.

Gametes with 23 *N. Tabacum* chromosomes and one or more chromosomes of *N. glutinosa* must not necessarily be substitution gametes. They become

substitution gametes only if one of the *N. glutinosa* chromosomes can take over the function of the missing *N. Tabacum* chromosome, as in the mosaic resistant race described above (table 2) or as in the one produced by HOLMES. Plants resulting from the fertilization of such ovules with normal tobacco

TABLE 3

Progeny from cross pentaploid×N. Tabacum.

POPULATION 45-241 PLANT NO.	CELLS ANALYZED	CELLS SHOWING 24II AND ADDITIONAL UNIVALENTS	23II	22II	21II	MOST FREQUENT CONFIGURATION
1	13	0	12	1	—	23II+7I
2	7	6	1	—	—	24II+1I
3	7	7	—	—	—	24II+2I
4	7	7	—	—	—	24II+5I+1 fragment
5	14	14	—	—	—	24II+2I
6	8	7	1	—	—	24II+2I
7	15	11	4	—	—	24II+1I
8	7	4	3	—	—	24II+1I
9	8	3	5	—	—	23II+2I
10	6	6	—	—	—	24II+1I
11	11	9	2	—	—	24II+3I+1 fragment (?)
12	19	16	1	2	—	24II
13	9	9	—	—	—	24II+5I
14	5	5	—	—	—	24II+3I
15	9	7	2	—	—	24II+3I
16	8	8	—	—	—	24II+1I
17	9	8	1	—	—	24II+4I
18	11	11	—	—	—	24II+3I
19		not studied				
20	7	7	—	—	—	24II+5I
21	8	8	—	—	—	24II+4I
22	9	9	—	—	—	24II
23	22	14	5	2	1	24II+2I
24	7	7	—	—	—	24II+3I
25	8	7	—	—	1	24II+2I
26	12	9	3	—	—	24II+4I
27	10	0	10	—	—	23II+4I
28	7	0	7	—	—	23II+3I
29	8	8	—	—	—	24II+2I
30	10	6	4	—	—	24II+2I
31	6	5	1	—	—	24II+3I

pollen are substitution heterozygotes, with a homologous non-conjugating pair consisting of a *glutinosa* and a *Tabacum* chromosome. Upon selfing and selection for a factor contained in the *N. glutinosa* chromosome, homozygotes with a substituted *N. glutinosa* pair will be secured.

Although no further progeny has yet been grown from this particular population (po. 45–241) described in the preceding lines, an actual substitu-

tion race was obtained in the fashion outlined below. This occurred in the offspring from the cross tetraploid (that is, 8x) *N. Tabacum* var. Cuba×*N. glutinosa*. "Cuba" is another white-flowered tobacco. A pentaploid plant from this cross (45–095p8), which was pink because of the presence of the *N. glutinosa* factor, was pollinated with Cuba pollen. In the progeny (45–383) white and pink segregants were obtained. Normal white Cuba was pollinated with pollen from one of the pink segregants, and a segregation of 1:1 for pink and white flowers resulted (populations 44–44, 44–65). The actual numbers were 27 pink:31 white ($P > .5$) from 75 seeds. The pink parent, therefore, was heterozygous and not trisomic for the pink factor. Transmission of an extra chromosome through the pollen is considerably lower than 50 percent (unpublished data). Pollen transmission for the members of a non-conjunctive pair, however, is 50 percent for each member. Pollen grains with only 23 chromosomes, which would result from lagging of the unassociated chromosomes, very rarely function in tobacco (CLAUSEN and CAMERON 1944). Such pollen grains, which would not carry the pink factor, could therefore not disturb a 1:1 ratio. A very few grains, on the other hand, might contain both unassociated chromosomes and add to the frequency of pink plants.

The 1:1 ratio indicated that a substitution heterozygote was obtained in the immediate progeny of a pentaploid plant and that the substitution occurred in meiosis in this plant, as postulated above. The results of further crosses corroborated the assumption that a substitution actually had occurred. A heterozygous plant of the second backcross population was selfed. The result was 24 white and 17 pink plants with both a low and high pollen fertility in either class. High pollen fertility indicated the disomic, homozygous character of the plants exhibiting it, low pollen fertility the monosomic or heterozygous condition.

One of the pink plants was monosomic with 23II+1I (44–82p8). When it was used as pollen parent in a cross with white, 23 pink and one unexpected white offspring were obtained. When selfed this monosomic gave only pink progeny. Some sibs were monosomics, as indicated by their low pollen fertility, whereas two were highly fertile. One of these fertile plants showed 24II (45–306p8). This latter plant then represents a homozygous substitution product. The monosomic parent (44–82p8) was presumably hemizygous for the pink factor.

At least one of the pink sibs of 44–82p8—namely, 45–245p5—was a homozygous substitution plant with 24II, since eight metaphases were studied and showed uniformly 24II, and the tetrad stage showed great regularity, with microcytes in only six out of 55 tetrads. These data indicate that a *glutinosa* chromosome was substituted for one of the chromosomes of the white Cuba tobacco and that this substitution happened during meiosis in the pentaploid *N. Tabacum-Tabacum-glutinosa* parent.

The data of HOLMES (1938) indicate that his mosaic resistant race was obtained through a mechanism similar to that described above for pink Cuba—that is, by substitution of a *glutinosa* chromosome for one in the *Tabacum* complement in the meiosis of the pentaploid. This is demonstrated in table 4,

which was derived from the data in table 1 of HOLMES' paper. The plant obtained by backcrossing the pentaploid and used for further selfing in his "Samsoun line" was a heterozygote and not a trisomic. This is indicated by the fact that it yielded upon selfing homozygous 24-paired resistant plants and also by the ratios obtained. Here again, then, we have a case of substitution in macrospore formation similar to the pink Cuba described above.

TABLE 4

The origin of mosaic resistant Holmes Samsoun.

RESISTANT		NON-RESISTANT	
			AMPHIDIPLOID *N. Tabacum-glutinosa*×*N. Tabacum*
132	:	0	Pentaploid×*N. Tabacum*
310	:	357	Heterozygote×self
324	:	110	Homozygote (selected)×self
399	:	0	True breeding 24-paired Holmes Samsoun tobacco

SUMMARY

The transfer of genes from one species to another frequently is impossible because of failure of the chromosomes to conjugate and to cross over in the hybrid. Substitution of a pair of chromosomes, where feasible, is a means of overcoming this difficulty. Such substitution may occur in plants which have one or more *N. glutinosa* chromosomes in addition to two full sets of *N. Tabacum* chromosomes. In these plants meiotic irregularities such as nonconjunction between the members of a *Tabacum* pair or trivalent formation between a tobacco pair and a *glutinosa* univalent may result in the production of sporocytes containing only 23 chromosomes of tobacco, besides one or more *N. glutinosa* chromosomes. A homologous *N. glutinosa* chromosome may then take the place of a tobacco chromosome. The causative irregularities may be observed in the meiosis of trisomic plants with 24 pairs of tobacco and a single *N. glutinosa* chromosome, and their occurrence has been demonstrated indirectly from the metaphase configurations of a family of plants derived from a pentaploid with the constitution of 24 *N. Tabacum* pairs and 12 *N. glutinosa* univalents. Two cases of accomplished substitution were shown to have their origin in the megasporogenesis of pentaploid plants.

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PHENOGENETIC STUDIES OF THE HOMOEOTIC MUTANTS OF DROSOPHILA MELANOGASTER. IV. HOMOEOTIC AND "GROWTH RATE" GENES

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A PREVIOUS paper (VILLEE 1945b) gave the results of combining the homoeotic mutants, aristapedia, aristapedia-Bridges, and proboscipedia, with the "growth rate" genes, dachs, dachsous, four-jointed, and combgap. Proboscipedia showed no interactions with any of the growth rate genes, the tarsi and labial palp structures of the combined stock were no different from those of the genes acting singly. Dachsous, four-jointed, and combgap were found to decrease the expression of aristapedia, and combgap decreased the expression of aristapedia-Bridges. Dachs, dachsous, and four-jointed increased the expression of aristapedia-Bridges. Both aristapedia and aristapedia-Bridges decreased the number of segments per tarsus and the length of the individual segments in the tarsus when combined with dachs, dachsous, four-jointed, or combgap. A comparison of the effects of changes in temperature during development (VILLEE 1943, 1944, 1945a) with the effects of combination with growth genes suggested that the dachs gene acts by a differential retardation and the combgap gene by a differential acceleration of the developmental processes which produce the aristapedia antenna. This agreed with WADDINGTON'S (1943) conclusions based on an embryological study of the growth rate genes. Several of the results, the absence of interactions with proboscipedia, differences in the actions of dachs and four-jointed on the expression of aristapedia and aristapedia-Bridges, could not be explained. Since they might have been caused by modifying genes present in one or more of the stocks used, the experiments were repeated after isogenic stocks had been established. The study was extended to include another homoeotic mutant, bithorax-34e.

MATERIALS AND METHODS

An isogenic wild stock was obtained by the following breeding procedure, similar to that used by SCHWAB (1940); single-pair matings were used throughout:

$$\frac{ClB}{+}\,\frac{+}{+}\,\frac{+}{+}\ \text{♀} \times \frac{+}{Y}\,\frac{Cy}{Pm}\,\frac{H}{Sb\ In3RC}\ \text{♂}$$

$$F_1\ \frac{ClB}{+}\,\frac{Cy}{+}\,\frac{Sb\ In3RC}{+}\ \text{♀} \times \text{her father},\ \frac{+}{Y}\,\frac{Cy}{Pm}\,\frac{H}{Sb\ In3RC}$$

$$F_2\ \frac{ClB}{+}\,\frac{Cy}{+}\,\frac{Sb\ In3RC}{+}\ \text{♀} \times \frac{+}{Y}\,\frac{Cy}{+}\,\frac{Sb\ In3RC}{+}\ \text{♂ yields}$$

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$\frac{+}{+}\frac{+}{+}\frac{+}{+}$ ♀♀ and $\frac{+}{Y}\frac{+}{+}\frac{+}{+}$ ♂♂, among others.

Four lines of isogenic wild flies were obtained, one of which seemed to be distinctly superior in fertility and viability. This was used in all further breeding, and the other three lines were discarded. As SCHWAB pointed out, this procedure probably results in a perfectly isogenic stock, although crossing over may possibly occur in spite of the *ClB*, *Cy*, and *In3RC* inversions.

The stocks used in the study, aristapedia (*ss*^{a}, 3–58.5), aristapedia-Bridges (*ss*^{aB}), proboscipedia (*pb*, 3–47.7), bithorax (*bx*, 3–58.8), dachs (*d*, 2–31.0), four-jointed (*fj*, 2–81.0), dachsous (*ds*, 2–0.3), and combgap (*cg*, 2–71.1), were made isogenic by repeated matings with the isogenic wild stock as follows:

isogenic wild ♀ × mutant ♂
F_1 female × F_1 male
isogenic wild ♀ × F^2 mutant ♂, etc.

This procedure was repeated eight times for each stock, which, according to the calculations of BARTLETT and HALDANE (1935), should leave an average of 20.5 crossover units of introduced chromosome on each side of the gene selected for. Similar calculations show that if the procedure had been repeated 16 times instead of eight there still would have been an average of 11.7 crossover units of introduced chromosome on each side of the selected gene. Actually a considerably smaller amount of introduced chromosome was present in some of the stocks, because the selected gene was extracted from a multiple gene stock. Dachsous was extracted from a dachsous dumpy (*dp*, 2–13.0) stock, and therefore the total length of introduced chromosome was less than 13 crossover units. Combgap was extracted from a combgap curved (*c*, 2–75.5) stock, so there were less than 4.4 crossover units of introduced chromosome to the right of the *cg* gene. Four-jointed was extracted from a *fj px sp* stock (plexus, *px*, 2–100.5; speck, *sp*, 2–107.0).

After the more or less isogenic stocks had been established, all possible combinations of homoeotic and growth rate genes were obtained by making the proper matings, breeding the F_1 flies together and selecting the desired types from the F_2. Large numbers of each type of cross were made, since only one fly in 16 had the desired combination. A special procedure was necessary to set up a combination of combgap and proboscipedia, since the females of both are completely sterile. A female of the constitution *ClB*/+, *Cy*/*Pm*, *H*/*SbIn3RC* was crossed to an isogenic *cg* male, and the F_1 female (*ClB*/+, *Cy*/*cg*, *SbIn3RC*/+) was crossed to an isogenic *pb* male. The offspring of this mating which were phenotypically wild type were mated brother to sister (+/+, *cg*/+, +/*pb* ♀ × +/*Y*, *cg*/+, +/*pb* ♂), and flies combining combgap and proboscipedia were selected from the F_2. All the F_2 crosses which gave rise to the combinations studied were made at a temperature of 25.0±0.3°C, in half-pint culture bottles using the standard cornmeal-molasses- agar medium.

As controls to the flies showing the combination of homoeotic and growth genes, flies showing the homoeotic character alone emerging from the same bottle were classified and recorded. In this way whatever slight variations in

temperature, nutrition, and humidity affected the phenotype of the homoeotic-growth gene combination also affected the phenotype of the homoeotic gene alone.

RESULTS

The results of the study are summarized in tables 1 to 4. The present experiments using isogenic stocks in general confirm the results of the previous crosses in which non-isogenic stocks were used. A few dissimilar results were obtained which indicate the presence in the previous crosses of modifying genes. These will be discussed in detail below.

Effects on the tarsi

Each of the "growth rate" genes normally affects the development of the tarsi. Dachs and four-jointed reduce the number of tarsal segments from five to four and decrease the size of the individual segments. The tarsi of dachsous

TABLE 1

Effects on the leg tarsi of homoeotic mutants in combination with the "growth rate" genes.

STOCK	SEGMENTS PER TARSUS					TOTAL FLIES	MEAN NUMBER OF SEGMENTS PER TARSUS ± S.E.
	1	2	3	4	5		
d				65		65	4.00
d, ss^{a}	64	77	6			147	1.60±0.046
d, ss^{aB}		31	44	48		123	3.14±0.07
d, bx		2	41	48		91	3.51±0.057
fj				86		86	4.00
fj, ss^{a}		19	35	39		93	3.22±0.079
fj, ss^{aB}		3	31	41		75	3.50±0.067
fj, bx			36	53		89	3.60±0.052
ds					71	71	5.00
ds, ss^{a}			27	47	52	126	4.19±0.069
ds, ss^{aB}				15	67	82	4.81±0.043
ds, bx					98	98	5.00
cg			2	9	58	69	4.81±0.056
cg, ss^{a}		3	34	51	17	105	3.78±0.073
cg, ss^{aB}			4	41	48	93	4.47±0.059
cg, bx			1	13	76	90	4.83±0.043

flies are shorter than normal and slightly swollen but five-segmented. The tarsi of combgap flies are extremely variable, some are almost normal, others are very swollen and bent; the number of segments varies from two to five. These genes also affect wing shape and venation and the shape and size of eyes, antennae, and thorax.

The homoeotic genes aristapedia, aristapedia-Bridges and bithorax, but not proboscipedia, interact with the growth genes to decrease further the number of segments per tarsus and the size of the individual segments (table 1) (see fig. 2–20, VILLEE 1945b). The results of the present experiments with aristapedia and aristapedia-Bridges agree very closely with those obtained previ-

ously with non-isogenic stocks, indicating that the shortening of the tarsus is caused in both experiments by the ss^a and ss^{aB} genes and not by any associated modifying genes. Bithorax has a slight tarsus-shortening affect with dachs and four-jointed, but not with dachsous or combgap. With both dachs and four-jointed the order of effectiveness of the homoeotic genes in decreasing the size of the tarsus is $ss^a > ss^{aB} > bx$.

Effects on the antennae

Aristapedia and aristapedia-Bridges normally affect the development of the antenna so that it is transformed to some degree into a tarsus. The variations in the phenotype have been classified as types 1 to 8 (see VILLEE 1943,

TABLE 2

Effects on the phenotype of the antenna of combining "growth rate" genes with the aristapedia alleles.

STOCK	ANTENNA TYPE								TOTAL FLIES	CODE	MEAN TYPE ±S.E.
	1	2	3	4	5	6	7	8			
d, ss^a						18	37	92	147	751	7.51±0.058
ss^a						15	43	101	159	755	7.55±0.052
fj, ss^a				29	26	17	14	7	93	541	5.41±0.132
ss^a					1	13	36	44	94	731	7.31±0.077
ds, ss^a					21	21	40	44	126	674	6.74±0.097
ss^a						4	41	86	131	763	7.63±0.048
cg, ss^a			2	56	40	7			105	450	4.50±0.064
ss^a						23	30	79	132	743	7.43±0.067
d, ss^{aB}		37	62	22	2				123	292	2.92±0.066
ss^{aB}	29	44	13						86	181	1.81±0.093
fj, ss^{aB}	53	22							75	129	1.29±0.052
ss^{aB}	45	51	16						112	174	1.74±0.066
ds, ss^{aB}		25	43	12	2				82	289	2.89±0.081
ss^{aB}	31	23	7	1					62	165	1.65±0.095
cg, ss^{aB}	78	15							93	116	1.16±0.038
ss^{aB}	19	42	21	2					84	206	2.06±0.082

for figures). Type 1 has only a small proximal portion of the arista tarsus-like; type 8 has a complete four- or five-segmented tarsus with claws on the distal segment; the other types are intermediate. The basal segments of the antennae of the higher aristapedia grades are also changed and leg-like. Aristapedia-Bridges is the weaker allele and its phenotypes are usually types 1 to 3. The stronger allele, aristapedia, normally is phenotypically grade 8, 7, or 6.

The phenotypic results of combining growth genes with the aristapedia alleles are given in table 2. The system of coding used in earlier studies is again used to facilitate comparisons of the effects of the combinations on the phenotype of the antenna. The code number is the sum of the products of the percentage of flies of each type times the type number. Its value, therefore, ranges from 100 (if 100 percent of the flies are type 1) to 800 (if 100 percent of the flies are type 8). The code number is a weighted average permitting a single

number to represent the effect of a given treatment or genotype on the phenotypes of a whole group of flies. It is not strictly proper to calculate a true mean type, since the types are not quantitative measures with equal intervals between successive types. However, the mean type and its standard error were computed for each set of data as a rough measure of the variation within each group and of the significance of the difference between each combination stock and its control. These differences were further checked by the use of Chi-square tests, and all differences were found to be highly significant except that between *d, ss*^{a} and its *ss*^{a} control.

Dachsous, four-jointed, and combgap interact in development with aristapedia to decrease its expression—that is, to decrease the amount of the antenna which is altered and leg-like; dachs does not have this action. Four-jointed and combgap decrease the expression of aristapedia-Bridges; dachs and dachsous increase it. The results are similar to those obtained previously in each combination except *fj ss*^{aB}. The previous experiments showed an increase in the expression of *fj ss*^{aB} over *ss*^{aB} alone; the present experiments with isogenic stocks show a definite decrease. This suggests the presence in the *fj* stock used previously of one or more modifying genes which result in an increase in the aristapedia expression; these genes were removed by the breeding procedure which rendered the stocks isogenic.

Effects on the halteres

The normal effect of the bithorax gene is to transform the haltere and metanotum more or less into a wing and mesonotum. An extra thorax and scutellum covered with bristles and hairs occurs between the normal meso-

TABLE 3

Effects of the "growth rate" genes on the phenotype of bithorax.

STOCK	HALTERE TYPE				TOTAL FLIES	CODE	MEAN TYPE ±S.E.
	1	2	3	4			
d, bx	26	51	14		91	186	1.86±0.068
bx	2	46	33	3	84	245	2.45±0.066
fj, bx		31	48	10	89	276	2.76±0.068
bx	1	68	7		76	207	2.07±0.035
ds, bx		8	48	42	98	335	3.35±0.063
bx	7	37	24	2	70	230	2.30±0.082
cg, bx	23	38	27	2	90	209	2.09±0.084
bx		21	54	12	87	290	2.90±0.065

thoracic scutellum and the abdomen. The halteres are enlarged and vary from an inflated, sac-like structure to a flattened, wing-like plate with typical wing veins, hairs, and costal bristles. The haltere variations were classified previously (VILLEE 1945a) as type 1 (a more or less normal haltere, perhaps slightly larger than usual) to type 7 (a large, flat, wing-shaped structure with

four longitudinal veins). Only types 1 to 4 were found in the present study, in which the allele *bx-34e* was used. The variations in the size, shape, and number of bristles of the enlarged metanotum parallel those of the haltere.

It is not surprising that the growth genes should affect the expression of the bithorax haltere, since they affect the size and venation of the normal wing. A set of code numbers, similar to the ones for aristapedia, was calculated to compare the effects of the various growth genes on the phenotype of bithorax (table 3). Means and standard errors were computed to provide a rough measure of the significance of the differences between the combination stocks and their controls. Chi-square tests of these differences show them all to be significant. Dachs and combgap decrease the expression of bithorax—that is, cause the haltere to be less wing-like; four-jointed and dachsous increase its expression.

Effects on the mouth parts

In proboscipedia, the mouth parts are changed from the normal oral lobes to tarsus-like or antenna-like structures. The tip of the proboscis bears a pair of structures extending laterally which may be tarsus-like—segmented append-

TABLE 4

Effects of the "growth rate" genes on the phenotype of proboscipedia.

STOCK	MOUTH PARTS						TOTAL FLIES	t/a+t
	TARSI	ARISTAE	TARSI/ ARISTAE	TYPE 4	TYPE 5	SMALL STUMPS		
d, pb	11	8	6	16	19	5	65	.605
pb	19	10	8	16	36	17	106	.65
fj, pb	14	10	7	12	19	13	75	.61
pb	16	8	5	16	27	11	83	.65
ds, pb	31	10	4	17	29	16	107	.69
pb	14	7	9	11	33	18	92	.66
cg, pb	17	9	3	9	35	11	84	.67
pb	12	8	7	17	31	9	84	.63

ages covered with bristles and hairs and tipped with claws—or antenna-like—short appendages ending in a heavy plumose bristle resembling an arista—or intermediate. For convenience, the phenotypes were classified in six groups: tarsi (both appendages tarsus-like), aristae (both appendages arista-like), t/a (one side resembling a tarsus, the other an arista), type 4 (basal half of each appendage tarsus-like, distal half arista-like), type 5 (basal three-fourths tarsus-like, distal quarter arista-like), and stumps (small, unsegmented, bristle-covered protrusions). In a previous study (1944) the fraction t/a+t (sum of tarsus-like appendages/ sum of tarsus-like and arista-like appendages) was used to compare the effects of temperature treatments given to developing larvae. The fraction is computed as follows: t="tarsi"+$\frac{1}{2}$ "t/a"+$\frac{1}{2}$ "type 4" +$\frac{3}{4}$ "type 5"; a+t= "tarsi"+"aristae"+"t/a"+"type 4"+"type 5." The flies with small stumps in place of oral lobes are omitted from the calculation.

This same device was used in the present study to compare the effects of different genotypes on the development of the mouth parts (table 4).

The fractions computed from the four sets of controls were very similar—.65, .65, .66, and .63. Those computed from the combinations of the several growth genes with proboscipedia differed slightly from the controls: dachs and four-jointed caused a slight decrease in the fraction; dachsous and combgap produced a slight increase in the fraction. Chi-square tests used to compare each combination with its control show that the differences are not significant (p = between .10 and .20). The present experiments therefore confirm the results of the earlier study (VILLEE 1945b): there is no significant effect of the growth rate genes on the expression of proboscipedia.

Other effects

Several types of abnormalities in the development of parts of the body occurred rarely in this as in the previous study: the absence of one or more legs, the absence of a wing, and the doubling of wings, legs, or antennae. One *fj ss*^{aB} fly was found with its left wing altered to resemble a three-segmented tarsus. It was similar to some of the phenotypes of podoptera (GOLDSCHMIDT 1945).

DISCUSSION

Very little is known of the mechanisms by which mutant genes cause the alteration of developmental processes which result in the mutant phenotype. A beginning on this problem and a summary of the knowledge to date was made by GOLDSCHMIDT (1938). WADDINGTON (1943), investigating the development of the genes dachs, dachsous, four-jointed and combgap, which cause a shortening of the legs, found that the phenotypes of the adults may be considered as the effects of alterations in the relative growth of parts which occur before the time of puparium formation. The many phenotypic effects on legs, wings, and body all follow from this change in growth rate. Certain combinations of these genes lead to extreme disturbances in growth and abnormal histogenesis: the conversion of wings into body surface, the formation of arista-like spikes on the ends of the tarsi, the conversion of eyes into palps or antennae, and the formation of "shoulders" which protrude anteriorly from the thorax and overhang the head. WADDINGTON postulated that the abnormal histogenesis is produced by abnormal foldings of the imaginal discs and that the sequence of events was: alterations of growth→abnormal foldings of the imaginal discs→abnormal histogenesis. He concluded that combgap causes an overgrowth and dachs a lessening of growth during the larval period.

Earlier experiments on the effects of exposing the larvae of homoeotic stocks to high or low temperatures during development (VILLEE 1943, 1944, 1945a) showed that cold treatments, presumably by a differential retardation of development, shift the development of the aristapedia antenna toward a tarsus, shift the development of the proboscipedia mouthparts toward aristae, and decrease the expression of bithorax (shift the development of the bithorax haltere toward a haltere). Heat treatments presumably by a differential ac-

celeration of development, shift the development of the aristapedia antenna toward an arista, shift the development of the proboscipedia mouthparts toward tarsi, and increase the expression of bithorax (shift the development of the bithorax haltere toward a wing).

The present results corroborate the previous ones in every case but the combination of four-jointed and aristapedia-Bridges. This combination increased the expression of ss^{aB} in the former experiments but caused a decreased expression (shifted development toward an arista) in the present study. The

TABLE 5

Comparison of the effects of temperature treatments and "growth rate" genes on the phenotypes of homoeotic mutants.

	TEMPERATURE		"GROWTH RATE" GENES			
	HEAT	COLD	DACHS	FOUR-JOINTED	DACHSOUS	COMBGAP
ss^{a}	−	o	o	−	−	−
ss^{aB}	−	+	+	−	+	−
bx	+	−	−	+	+	−
pb	+	−	o	o	o	o

o = no change in expression.
+ = increase in expression.
− = decrease in expression.

effects on the leg tarsi were identical in the two studies. Apparently one or more modifying genes were present in the *fj* stock used previously which affected the antenna but not the development of the tarsus. These were eliminated in setting up the isogenic *fj* stock used this time.

A comparison of the effects of temperature treatments and growth rate genes on the phenotypes of the homoeotic mutants (table 5) reveals the following: dachs has the same effect on the phenotypes of each of the homoeotic mutants as do cold treatments. Four-jointed affects the phenotypes of each of the homoeotic mutants similarly to heat treatments. The effects of dachsous on the phenotypes of the homoeotic mutants resemble those of heat treatments except in the combination *ds* ss^{aB}, and the effects of combgap on the phenotypes of the homoeotic mutants resemble those of heat treatments except in the combination of *cg bx*. Thus a comparison with the effects of temperature treatments suggest that dachs causes a decrease in the rate of development, and combgap, dachsous, and four-jointed cause an increase in the rate of development at some particular point.

The results of these experiments confirm the previous finding that, despite similar phenotypes and apparently similar modes of development, the genes *d* and *fj* have opposite effects on the development of the homoeotic genes. The possibility remains that one or more modifying genes may be linked very closely with *d* or *fj* and not have been eliminated from the stock by the breeding procedure employed, but this is unlikely.

The tarsi of aristapedia-Bridges flies are shortened by exposing mid or late larvae to X-rays (VILLEE 1946) as well as by exposing them to low temperatures (VILLEE 1943). The stock therefore has some tarsus-shortening factor (probably the aristapedia gene itself) which under normal conditions is below the effective threshold. Changes in the physical environment during development—for example, exposure to X-rays or to low temperatures—or changes in the genetic environment such as the addition of any of the growth rate genes, alter conditions so that the threshold is exceeded and the tarsi are shortened.

The effects of the growth genes on aristapedia, aristapedia-Bridges, and bithorax may be explained by GOLDSCHMIDT'S (1938, 1940) hypothesis of the action of the homoeotic mutants in producing their effects. GOLDSCHMIDT postulated that all the imaginal discs have identical potencies early in development but that they become mature (competent) at different developmental stages. Evocators for different modes of development are given off at different times in development. In the normal fly, each evocator is given off at the proper time to affect the proper disc which has just become mature and direct its development in the proper fashion. He postulated further that the homoeotic genes affect the time of maturation of particular discs so that they become competent earlier or later than normal and consequently are affected by a different evocator and develop in an abnormal fashion: an antenna develops as a tarsus, a wing develops as a haltere, etc. Exposing larvae of homoeotic flies to high or low temperatures causes an additional acceleration or retardation of development which is added to or subtracted from the acceleration or retardation produced by the homoeotic gene (see VILLEE 1944, 1945a for additional discussion). In a similar fashion, the growth genes, by speeding or slowing development in the critical stage when the discs are maturing, cause more or less of a particular disc to mature and be affected by a particular evocator.

The explanation of the absence of an effect of the growth genes on the phenotype of proboscipedia may lie in a difference in the time of maturation of the labial buds. The imaginal discs which give rise to the mouthparts may mature later than the others, later than the time of action of the growth genes. Temperature treatments are able to affect the development of proboscipedia as well as the development of the other homoeotics because they are given over a long period of time (one to eight days) and so include the effective period for proboscipedia. The growth genes may have rather sharply delimited periods of action which do not overlap that of proboscipedia but do coincide more or less with those of the other homoeotics.

Genetic studies of tetraltera (VILLEE 1942) revealed a similar interaction of the homoeotic gene with other genetic factors. The action of the tetraltera locus is suppressed by the inversions Glazed and Curly and enhanced by the inversions Moiré and Dichaete and the mutants eyeless and eyeless-Dominant. These factors may produce their effects by altering the rate of development and so upsetting the relations between the period of competence and the period of evocator release which exist in tetraltera, thus increasing or decreasing the extent to which the wing becomes haltere-like.

SUMMARY

Isogenic stocks of the growth rate genes dachs, dachsous, four-jointed, and combgap, and the homoeotic genes proboscipedia, aristapedia, aristapedia-Bridges, and bithorax were set up, all possible combinations of growth rate and homoeotic genes were made, and the phenotypes of the combinations noted.

Aristapedia and aristapedia-Bridges decrease the number of segments per tarsus and the length of the individual tarsal segments when combined with *d*, *ds*, *fj*, or *cg*. Bithorax decreases the number of segments per tarsus in combination with dachs and four-jointed but not in combination with dachsous and combgap.

Dachs has the same effect on the phenotypes of the homoeotic mutants as exposing the larvae of homoeotic flies to low temperature. Four-jointed, dachsous and combgap have an effect similar to that of exposing the larvae to high temperature. This corroborates and extends WADDINGTON's finding that dachs causes a lessening and combgap an increase in the rate of development during the larval period.

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MONOZYGOTIC AND DIZYGOTIC TWIN BIRTH FREQUENCIES IN THE TOTAL, THE "WHITE" AND THE "COLORED" U.S. POPULATIONS

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HUMAN twins are generally thought to be of two types, monozygotic or one-egg pairs and dizygotic or two-egg sets. The evidence on the basis of which this conclusion has been drawn is somewhat indirect, but it is convincing. That dizygotic twins exist is suggested by the fact that some pairs are of opposite sex and different in many other characteristics which are known to have a genetic basis. That monozygotic twins occur is attested to by incompletely separated pairs and by the fact that more same sex twins appear than what one would expect if all were dizygotic.

If twin sets are of two types, it is of interest to determine the relative birth frequencies of each kind. This can not be done empirically, at least not readily, but the proportion of each type can be estimated fairly accurately. This has been done for most foreign twin populations and to some extent for that of the United States. At least statements appear in publications regarding the relative proportions of each kind in the U.S. twin population. For example, NEWMAN, who has long been considered an authority on twins in America, published a book in 1940 entitled "Multiple Human Births," in which he writes as follows: "We conclude that in the United States about one fourth of all twins born are one-egg twins." AREY (1940) in his widely used embryological textbook "Developmental Anatomy" does not refer specifically to the U.S. twin population, but he comments as follows: "Statistically about one fourth of all twins are identifiable as of the single egg type."

The senior author of the present paper has never been able to obtain close agreement with the statements quoted above whenever he has roughly analyzed a limited set of U.S. twin data. He has always obtained monozygotic percentages which are somewhat higher. Since this has been true and since there is considerable reason for having correct U.S. monozygotic and dizygotic birth percentages available in the literature, it seems desirable to derive the best estimates which are possible from an extensive set of U.S. twin records and to publish these. This we are doing in the present paper. In addition we are presenting certain statistical analyses of twin percentages which bring out information which is of some interest.

The data which we have chosen for consideration are the twin birth frequencies of the U. S. Birth Registration Area from 1922 to 1936 inclusive. These years were selected because we have already analyzed certain other aspects of the U.S. population for this 15-year interval, and therefore possess data for those years which are required for a twin frequency analysis (STRANDSKOV 1945a, 1945b).

The method we are applying for an estimation of monozygotic and dizygotic

twin birth frequencies was suggested originally by WEINBERG in 1902. It is generally known as Weinberg's differential method. It is based on the theory that sex in man is genetically determined and that ♀♀, ♀♂ and ♂♂ sets of dizygotic twin births should appear in a population in proportions given by the square of the birth sex ratio of the total population.

The actual calculation of the frequency of dizygotic twin births consists of equating the observed number of ♀♂ sets to the expected percentage, and estimating in this manner the total number of dizygotic twins born. All excess

TABLE 1

The estimated percentages of monozygotic twin births in the total U.S. twin population, that is, in the "white" and the "colored" twin populations combined.

YEAR	TOTAL NO. OF SETS OF TWINS BORN	NO. OF TWIN BIRTHS OF THE DIFFERENT SEX COMBINATIONS			ESTIMATED PERCENTAGE OF MONOZYGOTIC TWIN BIRTHS BASED ON:		
		♀♀	♀♂	♂♂	SEX RATIO OF TOTAL POPULATION	SEX RATIO OF TWINS	50:50 SEX RATIO
1922	21,163	6,911	7,098	7,154	32.855	32.902	32.921
1923	21,444	7,033	7,113	7,298	33.594	33.641	33.660
1924	22,751	7,327	7,575	7,849	33.344	33.392	33.410
1925	21,531	7,063	6,919	7,549	35.665	35.711	35.730
1926	21,739	7,122	7,056	7,561	35.020	35.066	35.084
1927	25,752	8,390	8,439	8,923	34.393	34.440	34.459
1928	26,786	8,663	8,878	9,245	33.648	33.693	33.720
1929	26,489	8,673	8,823	8,993	33.319	33.365	33.384
1930	26,128	8,513	8,748	8,867	32.972	33.018	33.037
1931	25,067	8,062	8,507	8,498	32.058	32.106	32.126
1932	25,081	8,043	8,570	8,468	31.594	31.641	31.661
1933	24,990	8,098	8,471	8,421	32.137	32.185	32.205
1934	25,993	8,523	8,612	8.858	33.671	33.717	33.736
1935	25,197	8,122	8,397	8,678	33.286	33.329	33.349
1936	25,569	8,394	8,388	8,787	34.327	34.370	34.389
Total	365,680	118,937	121,594	125,149	501.883	502.576	502.871
Mean	24,379	7,929	8,106	8,343	33.459	33.505	33.525

same sex twins are considered monozygotic. With the number of dizygotic and monozygotic twin births estimated, the percentage of each type is, of course, easily obtained.

In table 1 are presented the total number of twin sets born each year from 1922 to 1936 inclusive, the number of ♀♀, ♀♂ and ♂♂ pairs, and, in the first of the last three columns of the table, the estimated yearly percentages of monozygotic births based on the birth sex ratio of the total population. This sex ratio, as presented previously by STRANDSKOV (1945b), is 48.431 percent ♀ : 51.569 percent ♂.

An examination of this third from the last column of table 1 reveals that for each of the 15 yearly twin populations, the estimated percentage of monozy-

gotic births among all twin births is close to 33⅓ percent rather than 25 percent as given by NEWMAN, AREY and others. For the total twin population of 365,680 sets, for the 15 year interval, the estimated percentage is 33.43. Thus it is evident that it is much more accurate to say that about one-third of all U.S. twin births are monozygotic rather than one-fourth as generally quoted. The difference is sufficiently great to warrant a correction in standard reference and textbooks. GREULICH (1934) and HAMLETT (1935) obtained somewhat similar percentages, but they did not emphasize the discrepancy and did not

TABLE 2

The estimated percentages of monozygotic twin births in the "white" U.S. twin population.

YEAR	TOTAL NO. OF SETS OF TWINS BORN	NO. OF TWIN BIRTHS OF THE DIFFERENT SEX COMBINATIONS			ESTIMATED PERCENTAGE OF WHITE MONOZYGOTIC TWIN BIRTHS BASED ON:		
		♀♀	♀♂	♂♂	SEX RATIO OF TOTAL POPULATION	SEX RATIO OF TWINS	50:50 SEX RATIO
1922	18,947	6,176	6,304	6,467	33.388	33.435	33.456
1923	19,143	6,298	6,298	6,547	33.133	34.180	34.200
1924	20,263	6,552	6,659	7,052	34.205	34.250	34.274
1925	19,460	6,412	6,173	6,875	36.490	36.536	36.557
1926	19,598	6,425	6,309	6,864	35.550	35.595	35.616
1927	22,581	7,334	7,357	7,890	34.773	34.817	34.839
1928	23,052	7,499	7,555	7,998	34.383	34.431	34.453
1929	22,647	7,422	7,504	7,721	33.665	33.709	33.731
1930	22,123	7,224	7,316	7,583	33.793	33.838	33.861
1931	21,095	6,782	7,074	7,239	32.860	32.908	32.932
1932	21,056	6,790	7,102	7,164	32.471	32.518	32.542
1933	20,741	6,721	6,926	7,094	33.147	33.190	33.214
1934	21,590	7,135	7,054	7,401	34.585	34.632	34.655
1935	21,279	6,911	6,983	7,385	34.301	34.344	34.367
1936	21,655	7,104	7,059	7,492	34.736	34.782	34.805
Total	315,230	102,785	103,673	108,772	512.480	513.165	513.502
Mean	21,015	6,852	6,911	7,251	34.165	34.211	34.233

present a detailed analysis. NEWMAN'S estimate is based on the data of NICHOLS pubiished in 1907.

Although WEINBERG suggested that the sex ratio of the population as a whole should be used as a base in the estimation of monozygotic and dizygotic twin birth frequencies, some investigators have argued that it is more appropriate to use the birth sex ratio of the twin population itself. This sex ratio could be sufficiently different from that of the total population to give significantly different estimated percentages. To find out whether or not this is true for the U.S. twin population, we have estimated the monozygotic birth percentages based on this ratio. For the total twin population in question the birth sex

ratio is 49.151 percent ♀ : 50.849 percent ♂. The estimated monozygotic percentages are given in the second from the last column of table 1. From an inspection of this column it will be seen that these percentages differ very little from those derived by using the sex ratio of the population as a whole. In fact, the mean of the two columns are so similar that for all practical purposes it is immaterial whether one employs the birth sex ratio of the total U.S. population or the birth sex ratio of the twin population itself.

Another possible sex ratio to use is the theoretically expected 50:50 ratio. It has been widely employed by investigators, because it is the easiest and the simplest to apply. In the last column of table 1 are shown the monozygotic

TABLE 3

The estimated percentages of monozygotic twin births in the "colored" U.S. twin population.

YEAR	TOTAL NO. OF SETS OF TWINS BORN	NO. OF TWIN BIRTHS OF THE DIFFERENT SEX COMBINATIONS			ESTIMATED PERCENTAGE OF "COLORED" MONOZYGOTIC TWIN BIRTHS BASED ON:	
		♀♀	♀♂	♂♂	SEX RATIO OF TOTAL POPULATION	SEX RATIO OF TWINS AND 50:50 RATIO
1922	2,216	735	794	687	28.294	28.339
1923	2,301	735	815	751	29.118	29.161
1924	2,488	775	916	797	26.326	26.367
1925	2,071	651	746	674	27.909	27.958
1926	2,141	697	747	697	30.173	30.220
1927	3,171	1,056	1,082	1,033	31.725	31.757
1928	3,734	1,164	1,323	1,247	29.084	29.138
1929	3,842	1,251	1,319	1,272	31.286	31.338
1930	4,005	1,289	1,432	1,284	28.439	28.489
1931	3,972	1,280	1,433	1,259	27.795	27.845
1932	4,025	1,253	1,468	1,304	27.006	27.056
1933	4,249	1,377	1,545	1,327	27.230	27.277
1934	4,403	1,388	1,558	1,457	29.185	29.230
1935	3,918	1,211	1,414	1,293	27.769	27.820
1936	3,914	1,290	1,329	1,295	32.039	32.090
Total	50,450	16,152	17,921	16,377	433.378	434.085
Mean	3,363	1,077	1,195	1,091	28.892	28.939

estimates based on this ratio. By examining these percentages it will be apparent that they also are very close to those obtained by using the birth sex ratio of the population as a whole. In fact they are so close that the general usage of a 50:50 ratio is justified. This statement holds, at least, for the estimation of the percentage of monozygotic births within U.S. twin populations.

It may be desired by some to have on hand the estimated yearly dizygotic percentages. We have these available and have made use of them in our statistical analyses, but we shall not publish them in full. They can, of course,

be obtained directly by subtracting the estimated yearly percentages of monozygotic births from 100 percent. For the total 15 year period the estimated dizygotic percentage based on the sex ratio of the population as a whole is 66.57.

The monozygotic percentages presented in table 1 are those for the total U.S. twin population. From a genetic point of view it is of interest, to find out if racial differences exist. Twin birth data are not presented in the U.S. census

TABLE 4

Estimated monozygotic and dizygotic birth percentages among U.S. twin births based on the sex ratio of all births in the population in question. The data are for the years 1922 to 1936 inclusive.

POPULATION	TOTAL NO. OF TWIN SETS BORN DURING 15 YEAR INTERVAL	ESTIMATED PERCENTAGES OF	
		MONOZYGOTIC SETS	DIZYGOTIC SETS
Total	365,680	33.432	66.568
"White"	315,230	34.156	65.844
"Colored"	50,450	28.908	71.092

records for all the racial sub-groups of the U.S. population for the period under consideration, but they are available for what the Bureau of the Census calls the "white" and the "colored" U.S. populations. The "white" population probably consists almost exclusively of members of the Caucasoid stock, but the "colored" population is a mixture of Negroid, Mongoloid and Caucasoid. It is, however, largely Negroid. Therefore an estimate of its monozygotic birth frequency will be fairly representative of the U.S. Negroid stock.

The estimated percentages of monozygotic births in the "white" U.S. twin population from 1922 to 1936 inclusive are shown in table 2. Those for the "colored" are presented in table 3. As before, the estimated yearly percentages of dizygotic births are not published, but the estimates for the total 15 year period are listed in table 4.

With the yearly monozygotic and dizygotic percentages estimated for the total, the "white" and "colored" U.S. twin populations, it is of interest to compare the means of these different populations to see whether or not they are significantly different. This can be done by applying the standard formula for a test of the significance of the difference between two means:

$$t = \frac{\Delta}{\sqrt{\frac{\Sigma(x_1 - \bar{x}_1)^2 + \Sigma(x_2 - \bar{x}_2)^2}{N_1 + N_2 - 2} \times \frac{N_1 + N_2}{N_1 N_2}}}$$

The t values for the pairs of percentage means which have been compared, together with the probabilities of the t values, are shown in table 5. If we examine this table, we find that significant differences between the means of some of the populations do occur. Of particular interest are the significant differences between the means of the "white" and the "colored" twin populations. The

monozygotic percentage of the "white" twin population is significantly higher than that for the "colored," and, as follows, the dizygotic percentage of the "white" twin population is significantly lower than that for the "colored."

In a previous paper (STRANDSKOV 1945a) we showed that the percentage of twin pregnancies among all pregnancies is significantly higher in the U.S.

TABLE 5

The t values and their probabilities obtained by comparing the means of the estimated percentages of monozygotic births among twin births in the total, the "white," and the "colored" U.S. populations from 1922 to 1936 inclusive.

POPULATIONS COMPARED*	*t* VALUE	PROB. LESS THAN
"White" vs. Total	1.82	.1
Total vs. "colored"	8.61	.001
"White" vs. "colored"	10.12	.001

* In each instance the population with the larger mean percentage is shown first.

"colored" population than it is in the "white." (The respective percentages for the 15 year interval in question are 1.433 and 1.129.) With the number of monozygotic and dizygotic births estimated for each year for both the "white" and the "colored" population, it is, of course, possible to calculate the monozygotic and dizygotic pregnancy percentages for each population and, consequently, to decide whether the higher twin pregnancy percentage in the "colored" population is due to more monozygotic pregnancies, to more dizy-

TABLE 6

The estimated percentage of monozygotic and dizygotic twin births or pregnancies among all pregnancies in the total, the "white," and the "colored" U.S. population from 1922 to 1936 inclusive.

POP-ULATION	TOTAL NO. OF SINGLE AND PLURAL PREGNANCIES	ESTIMATED MONOZYGOTIC PREGNANCIES		ESTIMATED DIZYGOTIC PREGNANCIES	
		NO.	%	NO.	%
Total	31,487,413	122,253	.388	243,427	.773
"White"	27,923,410	107,669	.386	207,561	.743
"Colored"	3,564,003	14,584	.409	35,866	1.006

gotic, or to more of both types. The monozygotic and dizygotic pregnancy percentages for the total, the "white," and the "colored" U.S. populations for the 15 year interval are shown in table 6. The t values obtained from a comparison of the means of the yearly pregnancy percentages of the different populations are presented in table 7.

If we examine tables 6 and 7, we find that both the monozygotic and the dizygotic twin pregnancy percentages are significantly higher for the "colored" population than they are for the "white." Hence we may conclude that the

significantly higher total twin pregnancy frequency in the "colored" population is due, not only to more dizygotic twin pregnancies, but also to more monozygotic.

Since we have found racial differences in the percentage of monozygotic pregnancies as well as in the percentage of dizygotic pregnancies, it is appropriate to consider the probable causes of these differences. Are the responsible factors environmental, genetic, or in part both types? It is not possible on the

TABLE 7

The t values and their probabilities obtained by comparing the means of the estimated percentages of monozygotic and dizygotic pregnancies among all pregnancies in the total, the "white" and the "colored" U.S. populations from 1922 to 1936 inclusive.

POPULATIONS COMPARED*	t VALUE	PROB. LESS THAN
A. Monozygotic percentages		
Total vs. "white"	.64	.6
"Colored" vs. total	2.51	.02
"Colored" vs. "white"	3.46	.01
B. Dizygotic percentages		
Total vs. "white"	4.12	.001
"Colored" vs. total	22.56	.001
"Colored" vs. "white"	26.80	.001

* In each instance the population with the larger mean percentage is shown first.

basis of the present data to come to any final decision with respect to this question, but it is possible to point out a few facts which present some evidence in favor of one point of view or another. If we compare the observed variance of each distribution of 15 yearly percentages of monozygotic and dizygotic pregnancies in both the "white" and the "colored" populations with the variance expected due to chance, we find that for all of the distributions considered, the observed variance is significantly larger than the variance expected due to chance. This is shown by the F values in table 8. (For a discussion of the statistics F see FISHER 1935 and SNEDECOR 1939). Since the observed variance is significantly larger for the more or less racially homogeneous populations, as well as for the mixed groups, it seems logical to conclude that environmental factors can and do affect the incidence of occurrence of both monozygotic and dizygotic twinning in man. It does not seem likely that genetic factors varied sufficiently within the "white" or the "colored" population during the 15 year interval to be responsible for all or even most of the excessive observed variance.

Although we are inclined to think that we have evidence which suggests that environmental factors can and do affect the incidence of occurrence of both monozygotic and dizygotic twinning in man, we are not of the opinion that environmental factors are responsible for all of the observed racial differences in monozygotic and dizygotic pregnancy percentages. That dizygotic twinning in man has some genetic basis is generally accepted on the basis of

pedigree analyses. Hence it would not be surprising to find a racial difference as large as that observed due to heredity. Whether or not all of the observed racial difference in dizygotic twinning is due to genetic factors, our data do not permit us to say, but we are inclined to think that much of it is. That monozygotic twinning in man has some genetic basis is less certain. In fact most investigators question it. We do know, however, that monozygotic twinning or quadrupling among lower animal forms such as the armadillo is genetically determined. Hence it would not be unreasonable to suspect that monozygotic

TABLE 8

The F values or the extent to which the observed variance of each of twelve different distributions of monozygotic and dizygotic percentages is greater than the variance expected due to chance.

DISTRIBUTIONS ANALYZED	F* VALUE	PROB. LESS THAN
A. Monozygotic births among:		
1. twin births	13.20	.01
2. "white" twin births	9.86	.01
3. "colored" twin births	4.95	.01
4. pregnancies in total population	8.01	.01
5. pregnancies in "white" population	5.81	.01
6. pregnancies in "colored" population	4.88	.01
B. Dizygotic births among:		
1. twin births	13.20	.01
2. "white" twin births	9.86	.01
3. "colored" twin births	4.95	.01
4. pregnancies in total population	12.65	.01
5. pregnancies in "white" population	7.55	.01
6. pregnancies in "colored" population	2.72	.01

* In each instance the observed variance is greater than the variance expected due to chance. (F. 1.69 has a probability of .05 and F. 2.07 a probability of .01.)

twinning in man might be partially influenced by heredity. Our observed racial difference in the incidence of occurrence of monozygotic twinning is relatively small and could presumably be due entirely to racially different environmental factors, but we are of the opinion that it is due in part to a rôle played by heredity.

SUMMARY

The proportions of monozygotic and dizygotic twin births among all twin births in the total, the "white," and the "colored" U.S. populations from 1922 to 1936 inclusive, are estimated. It is found that the respective monozygotic percentages are 33.46, 34.17, and 28.89. The racial difference is statistically significant.

Although the "colored" population has a significantly lower proportion of monozygotic births among twin births, it has a significantly higher percentage of twin births among all births. The percentages are 1.43 for the "colored" population and 1.13 for the "white."

The greater percentage of twin births among all births in the "colored" population is due not only to more dizygotic births but also to more monozygotic. The respective "colored" and "white" dizygotic percentages are 1.01 and 0.74. The respective monozygotic percentages are 0.41 and 0.39. Both of these racial differences are statistically significant.

The observed variance of each distribution of 15 yearly twin percentages is compared with the variance expected due to chance. For all of the twelve distributions considered, the former is found to be significantly larger than the latter. This suggests that environmental factors can and do affect the incidence of occurrence of both monozygotic and dizygotic twinning in man.

Although it is thought that environmental factors do affect the incidence of occurrence of dizygotic twinning in man, the conclusion is reached that much of the observed racial difference in the percentage of dizygotic pregnancies among all pregnancies is due to hereditary differences.

The observed racial difference in the percentage of monozygotic pregnancies among all pregnancies is small and could presumably be due entirely to racial environmental differences. Yet we are inclined to think that the data suggest a partial rôle played by hereditary factors.

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SUMMARIES FROM FORTHCOMING PAPERS

KAUFMANN, BERWIND P. *Modification of the frequency of chromosomal rearrangements induced by X-rays in Drosophila. III. Effect of supplementary treatment at the time of chromosome recombination.*—Received March 7, 1946.— Eggs deposited by females of *Drosophila melanogaster* that had been inseminated by males previously exposed to 4000 roentgens of X-rays were kept either at 18°C or at 28°C, or exposed to near infrared radiation (x ca. 10,000 Å) during the period of syngamy and early cleavage in order to measure the effect of these agents on chromosome recombination.

The frequency of chromosomal rearrangements detected by analysis of salivary gland chromosomes was higher in the group exposed to the near infrared radiation than in the groups kept at 18° or 28°C (table 1). The χ^2 test indicates that the values obtained following the use of near infrared are significantly higher than in the large group of about 1800 pairs of glands secured in a series of experiments in which temperatures of 18°C or 28°C were maintained during syngamy and early cleavage stages.

Although it increases the temperature within the organism during the period of exposure, the effective action of near infrared radiation in facilitating recombination among the breaks induced by the ionizing radiation is probably not limited to an extension of the range of temperature within which fertility and viability may be maintained in Drosophila, but may depend on selective action on certain cellular components.

CASPARI, ERNST. *On the effects of the gene a on the chemical composition of Ephestia kühniella Zeller.*—Received March 21, 1946—In *aa* Ephestia, both in imagoes and larvae, the tryptophane content is increased as compared to a^+a^+ Ephestia. a^+a heterozygotes have the same tryptophane content as a^+a^+ animals. In F_2, *aa* material has the increased tryptophane content of the original *aa* strains, indicating that the increase in tryptophane content is caused by the gene *a*. No differences in tryptophane content were found between *bw* and *v bw* Drosophila. In *aa* material, most or all of the increased amount of tryptophane is contained in the proteins. The relative amount of tryptophane in *aa* proteins is increased. No difference in protein content between the two strains was found. a^+a^+ proteins are distinguished from *aa* proteins by a pink coloration. In *aa* animals, the ether-extractable materials are reduced in amount as compared to a^+a^+ animals.

It is concluded that in *aa* animals the oxidation of tryptophane to kynurenin is inhibited. This may be caused either by a direct inhibition of the oxidation process, the proteins being changed secondarily, due to an increased amount of tryptophane available, or primarily by a different structure of the proteins causing tryptophane not to be available for kynurenin production. It is concluded that as a result of the gene substitution $a^+ \rightarrow a$ the biochemical setup of the cell is profoundly changed.

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